HIGH YIELDING TROPICAL ENERGY CROPS FOR BIOENERGY PRODUCTION: EFFECTS OF CROP MATURITY, PLANT COMPONENTS, HARVEST YEARS AND LOCATIONS ON BIOMASS COMPOSITION AND SUBSEQUENT BIOGAS YIELD

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To my family

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ABSTRACT

Bioenergy and biobased products generation from C_4 perennial lignocellulosic feedstocks have attracted significant research interest due to their relatively high biomass yield at low agricultural inputs. However, biomass yield and composition of lignocellulosic energy crops vary with the species, crop maturity, crop components, and agro-climatic conditions. Such variation in the composition of the feedstock is believed to have a significant effect on the conversion process as well as on the yield and quality of the end products.

Thus, this study examined the compositional changes of Napier grass (*Pennisetum purpureum*) with respect to maturity (2, 4, 6, and 8 months age), and its effect on anaerobic digestion under three different biomass size regimes (6, 10, and 20 mm). This study also investigated the composition of different components (stems and leaves) of two energy crops [Energycane (*Saccharum hybrids*) and Napier grass] collected across the three locations and three years, and their effects on anaerobic digestibility.

Significant changes in plant composition were observed with crop maturity. The methane yields were higher for the biomass harvested at younger stages of maturity. For all ages, feedstock passed through a 6-mm sieve resulted in significantly higher methane yields compared to biomass passed through 10 and 20 mm sieves. Additionally, fiber digestibility was highest for the 2-month old harvest biomass and was lowest for the 8-month old harvest biomass.

Higher fiber content was found in the leaves of Energycane than stems, but the fiber content was higher in stems than leaves of Napier grass. Furthermore, the top leaves

and top stems of the Energycane resulted in higher specific methane yield compared to the bottom stems and bottom leaves. In Napier grass, however, specific methane yield was higher from leaves than stems. Between the crop types, for all locations and harvest years, fiber content was higher in Napier grass than Energycane. For all locations, Energycane had higher specific methane yield than Napier grass.

Depending on crop maturity, crop type, and plant components, energy crops differ significantly in composition and in specific methane yield, and require either different pretreatment conditions or conversion technologies for effective utilization of complete biomass.

PREFACE

Research work based on objective 1 was published in the journal of *Bioresource Technology* (***Surendra, K.C.,*** *Khanal, S.K., 2015. Effects of crop maturity and size reduction on digestibility and methane yield of dedicated energy crop. Bioresource Technology 178, 187-93*) and content was used in this dissertation with the permission from the publisher

The literature on anaerobic digestion of energy crops and anaerobic digestion-based biorefinery were critically reviewed in detail in the following publications, and thus the literature review was not included in this dissertation. The following publications are refered for the critical review on anaerobic digestion of energy crops and anaerobic digestion-based biorefinery:

- *Sawatdeenarunat, C.,* ***Surendra, K. C.,*** *Takara, D., Oechsner, H., and Khanal, S. K. (2015). Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. Bioresource Technology, 178, 178-186*
- ***Surendra, K. C.,*** *Sawatdeenarunat, C., Shrestha, S., Sung, S., & Khanal, S. K. (2015). Anaerobic digestion-based biorefinery for bioenergy and biobased products. Industrial Biotechnology, 11(2), 103-112.*

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AD	Anaerobic digestion
TS	Total solids
VS	Volatile solids
LCA	Life cycle assessment
GHGs	Greenhouse gases
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
ADL	Acid detergent lignin
ANOVA	Analysis of variance
HBT	Hohenheim biogas yield test
MJ	Mega joule
r	Correlation coefficient
CH_4	Methane

CHAPTER 1: INTRODUCTION

1.1 Background

With significant growth in global population [estimated to increase from 7.3 billion in 2015 to 9.7 billion in 2050 (United Nations, 2015)] and economy, the demand for energy is estimated to increase from 549 quadrillion Btu (QBtu) in 2012 to 815 QBtu in 2040, an increase of 48% compared to 2012 (EIA, 2016). On one hand, the current energy supply is heavily dependent on fossil fuels, which account for about 78% of global energy demand (REN21, 2016). On the other hand, dependency on fossil fuels may have significant impact on our energy security and, more importantly, environmental sustainability, including local/regional air quality, and greenhouse gas (GHG) emissions. For example, about two-thirds of the GHG emissions are estimated to be associated with the production and consumption of fossil-based energy resources (IEA, 2015). Thus, to address the issues associated with fossil fuels, research and development are exploring clean renewable energy technologies. Such efforts have already made renewable energy one of the world's fastest growing energy sources. For example, consumption of renewable energy, which accounted for 19.2% of global energy consumption in 2014, is estimated to increase by an average annual growth rate of 2.6% between 2012 and 2040 (EIA, 2016; REN21, 2016). Biomass (including traditional biomass), which currently contributes the largest share of renewable energy at over 50% of global renewable energy consumption (over 10% of global energy consumption) (REN21, 2016), is seen as an important component of the renewable energy mix (IEA, 2017). Currently biofuels are the major renewable transportation fuel, accounting for 4% or 134 billion liters of global road transportation fuel in 2015 and estimated to reach almost 4.5% by 2020 with an average annual growth rate of 2.5% between 2015 and 2020 (IEA, 2017). Currently biofuels are produced mostly from the first generation feedstocks (e.g., corn ethanol in the USA, sugarcane ethanol in Brazil, and rapeseed biodiesel in the European Union), which raises serious debate on use for food and/or feed versus fuel. Thus, to increase the share of bioenergy in the global energy supply while avoiding or minimizing the food and/or feed versus fuel issues, research efforts are being made to produce bioenergy/biofuel from non-food and/or feed feedstocks, such as lignocellulosic biomass, which is the most abundant bioresource on the earth with 200 billion metric ton availability per year (Ren *et al.*, 2009). Among various lignocellulose feedstocks, C_4 perennial energy crops have gained significant attention due to several benefits associated with perennial energy crops. For example, in addition to providing a consistent quality of feedstocks for bioenergy and biobased product generation, perennial energy crops offer environmental and ecological benefits, such as improving soil health, increasing carbon sequestration, reducing soil erosion and enhancing water conservation (Xue *et al.*, 2011; Sumiyoshi *et al.*, 2016).

One of the major challenges with lignocellulosic biomass as a feedstock for bioenergy production is the complex feedstock composition, unlike first generation feedstocks, which are mainly starch-based (corn, cassava, barley) and sugar-based (sugarcane, sugar beet), and are readily fermentable to ethanol with or without enzymatic hydrolysis. Thus, the pretreatment and enzymatic hydrolysis of lignocellulosic feedstocks are critically important and account for up to 29% of total equipment and 45 - 55% of operating costs for biofuel production (Valdivia *et al.*, 2016), while feedstock alone contributes about 38% of the operating costs (Gnansounou and Dauriat, 2010). Currently, the cost of biofuel production from lignocellulosic biomass is 2 - 3 fold higher (based on energy value) than petroleum fuels (Carriquiry *et al.*, 2011). Thus, cost-effective production of biofuels and biobased products from lignocellulosic feedstocks needs significant improvement in both biomass productivity and conversion efficiency (Sims *et al.*, 2010; Feltus and Vandenbrink, 2012).

Biomass composition significantly affects the conversion efficiency of feedstocks into biofuel and biobased products (Lee, 2007), and ultimately the yield and quality of the product of interest (Xue *et al.*, 2011). For example, ash content in feedstocks negatively affects most conversion processes (Aurangzaib *et al.*, 2016; Williams *et al.*, 2016). High ash content in feedstock reduces the effectiveness of the acid pretreatment during biochemical conversion (Weiss *et al.*, 2010). In thermochemical conversion, such as combustion, gasification and pyrolysis, high ash content in feedstocks produces fusible slag that fouls boilers, heat exchangers and machinery, which ultimately reduces the conversion efficiency and increases the maintenance cost of the plant (Weiss *et al.*, 2010; Aurangzaib *et al.*, 2016). For every 1% reduction in ash content in feedstocks, a 1 - 5% increase in bio-oil yield during pyrolysis of feedstocks has been reported (Fahmi *et al.*, 2008b; Carpenter *et al.*, 2014; Williams *et al.*, 2016). Similarly, lignin present in feedstocks deters enzyme/chemical access to cellulose and hemicellulose (Sun and Cheng, 2002) during biochemical conversion, while degradation products of lignin (furans and phenols) generated during thermochemical pretreatment of lignocellulosic feedstocks inhibit enzymatic hydrolysis and microbial fermentation during downstream processing (Klinke *et al.*, 2004). Since lignin has a higher heating value (26.7 MJ kg^{-1}

lignin) than the cellulosic fraction (17.3 MJ kg^{-1} cellulosic fraction) (Jenkins *et al.*, 1998), a higher lignin content favors thermochemical conversion. Increase in lignin content in the feedstocks has been reported to increase the bio-oil yield and average molecular weight of the bio-oil during pyrolysis (Fahmi *et al.*, 2008a).

It is equally important to examine changes in biomass composition, which are highly dependent on crop genotype, crop management, crop maturity, locations or environmental conditions, and plant parts (Lee, 2007; Waramit *et al.*, 2011). Current research on lignocellulosic biomass mainly focuses on biomass conversion to enhance the conversion efficiency to produce biofuels and biobased products. Since the composition of biomass governs the conversion efficiency, it is critically important to select the right crop and crop management practices for a particular location, and to fragment the biomass into different plant parts (leaves and stems) based on their composition. Such research will provide guidelines in selecting an appropriate conversion technology for efficient processing of the lignocellulosic biomass into diverse products of interest. For example, plant parts with low lignin content; but high ash and moisture content are not ideal for thermochemical conversion processes (combustion, pyrolysis, torrefaction, and gasification among others). Similarly, plant parts with high lignin content require high inputs of energy and chemicals for biochemical conversion.

Despite a plethora of biomass-to-bioenergy technologies proposed to date, however, anaerobic digestion (AD) - a technology extensively used in wastewater treatment - has emerged as one of the most energy efficient conversion processes (Swedish Government Official Report, 2007). The use of AD for electricity production, heating, and transportation has dominated the bioenergy production sector in many

European countries during recent years. There are more than 17,000 established and operating commercial biogas plants in the European region, and Germany alone boasts over 10,000 biogas facilities (European Biogas Association, 2015). The flexibility of AD in terms of feedstock use (from municipal and industrial organic wastes to agricultural and forestry residues, and dedicated energy crops) as well as the end-use applications of the produced biogas (combined heat and power generation (CHP) or methane upgrades for transportation fuel) (Amon et al., 2007) makes AD a highly attractive biomass-to-bioenergy conversion process among other competing technologies. In the context of AD, the reinforced structure of lignocellulosic biomass complicates the deconstruction of polysaccharides like hemicellulose and cellulose. Consequently, hydrolysis (the first step of the AD process) is often a rate limiting step (Andey et al., 1991). Moreover, as mentioned earlier, the composition of the energy crops varies with species, crop maturity, plant parts, environmental conditions, and their interactions. This natural variation in composition significantly affects both digestibility and overall methane yield during AD (Gunaseelan, 1997; Cherney et al., 1986). Current studies on the conversion of energy crops to methane focus primarily on different pretreatment methods (thermo-chemical, physical, biological or hybrid) but not much on the characteristics of the biomass itself (Li et al., 2012; Xie et al., 2011; Seppala et al., 2008). Though these studies have reported better methane yields through improved hydrolysis of biomass, the bioprocessing methods suggested are often too costly. Within this context, a clear understanding of biomass composition with respect to crop types, crop maturity, crop parts, environmental conditions and their interactions, and its subsequent implication on biomass digestibility for methane production is crucial to maximize the net energy yield from a given planted area.

Napier grass (*Pennisetum purpureum*) and Energycane (*Saccharum hybrids*) have attracted significant attention as promising feedstocks for tropical and subtropical regions of the world due to their efficient C_4 photosynthetic pathway (comparatively high biomass yield at low inputs, with better water and nutrient use efficiency), upright growth (facilitates efficient harvesting), and perennial nature (in addition to providing environmental and ecological benefits, requires less labor and input costs in crop management) (Na *et al.*, 2014b). Studies have reported higher yield of Energycane and Napier grass compared to other dedicated energy crops such as Switchgrass (*Panicum virgatum*), and Miscanthus (*Miscanthus spp.*). For example, depending on locations, cultivars, and crop management practices, the dry matter yields of Energycane and Napier grass have been reported to vary from 8 - 53 Mg ha^{-1} year^{-1} and 5 - 47 Mg ha^{-1} year^{-1} (Fedenko *et al.*, 2013), respectively. On the other hand, the average yields of Miscanthus and Switchgrass, respectively, have been reported to vary from 9 - 24 Mg ha^{-1} year^{-1} (McKendry, 2002; Fedenko *et al.*, 2013) and 1 - 23 Mg ha^{-1} year^{-1} (McKendry, 2002; Schmer *et al.*, 2009; Song *et al.*, 2014).

In recent years, performance (yield and quality) of candidate energy crops under different management practices has been extensively discussed elsewhere (Ansah *et al.*, 2010; Schmer *et al.*, 2012; Fedenko *et al.*, 2013; Knoll *et al.*, 2015; Na *et al.*, 2016; Cole *et al.*, 2017). To the best of our knowledge, there are limited studies on the quality or composition of tropical energy crops harvested across different years/seasons and locations. Moreover, only a few studies have examined the composition of the different parts of the tropical energy crops. An in-depth understanding of the effects of crop types, crop maturity, harvest years/seasons, locations, and plant parts on biomass yield and quality is critical for cost-effective conversion of lignocellulosic biomass into biofuel and biobased products (Schmer *et al.*, 2012). Detailed characterization of the plant parts of the energy crops helps in selecting the appropriate conversion technology (in downstream processing) to maximize conversion efficiency and ultimately the yield and quality of end products. Additionally, returning the plant parts, which are either lower in energy content or require more inputs (energy and chemicals) for processing into bioenergy and biobased products, into the soil could improve soil health and crop productivity, while reducing the cost of biomass conversion into biofuel and biobased products. Thus, the overall goal of this study was to examine the effects of crop maturity, locations, harvest years (and seasons for Napier grass), and plant parts (leaf and stem) on the composition of two dedicated perennial C_4 energy crops, Energycane and Napier grass, grown in Hawaii and to evaluate their anaerobic digestibility for biogas production.

1.2 Goals and Objectives

The overarching goal of this research was to study the effects of crop maturity, plant parts, locations and harvest years (and seasons for Napier grass) on the composition of the selected energy crops for biofuel productions. The specific objectives of the study were:

1. To evaluate the effects of maturity and size reduction on anaerobic digestibility of lignocellulosic biomass (Napier grass) for biogas production.
2. To investigate the fiber composition of plant parts of the selected energy crops harvested at three locations and years.

3. To examine the anaerobic digestibility of the selected energy crops harvested at three different locations for biogas production.

1.3 Hypotheses

1. The composition of the energy crop varies with the maturity.
2. The composition of energy crops varies with plant part, crop type, location and harvest year.
3. The variation in composition of energy crops affects their digestibility for biogas production.

CHAPTER 2: MATERIALS AND METHODS

2.1 Materials and Methods: Objective 1

2.1.1 Substrate

Ratooned Napier grass, grown at the Waimanalo Research Station (Waimanalo, HI, USA), was hand-harvested at growth stages of 2, 4, 6 and 8 months; Napier grass reaches full maturation at around 7 - 8 months. The hand-harvested biomass was then shredded using a commercial cutting mill (Vincent Corporation, Tampa, FL, USA) for initial size reduction and the resulting shredded material was further passed through a second laboratory cutting mill (Retch SM2000, Haan, Germany) with different screen sizes of 6 mm, 10 mm and 20 mm. The extruded biomass was analyzed for total solids (TS), volatile solids (VS), and fiber composition [i.e., Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Acid Detergent Lignin (ADL)]. The milled biomass was used for AD in this study.

2.1.2 Inoculum

The inoculum used in this experiment was taken from a 20 L inoculum reactor maintained in our laboratory. The inoculum reactors were fed with cattle manure and kept at a mesophilic condition (37 ± 1 °C). The reactor contents of the inoculum reactor were allowed to reach a stable neutral pH of 6.8 - 7.4 before being used as the inoculum in the digestion study. To minimize interferences from fibers inherently found in the manure-derived inoculum, the contents of the inoculum reactor was sieved using a #8 sieve (ASTM 2.36 mm, Thermo Fisher Scientific Inc., USA), and the collected liquid was used as primary inoculum for digestion tests. The TS and VS contents of the inoculum were

$5.2 \pm 0.10\%$ and $75.4 \pm 0.14\%$ (% TS), respectively. The inoculum was stored at 4 °C for about 3 - 4 days before being used in digestion tests.

2.1.3 Digestion test

Digestion study was conducted in a series of 2 L serum bottles (Friedrich and Dimmock, Inc., Millville, NJ, USA) with a working volume of 1.5 L. The substrate-to-inoculum ratio was maintained at a 1:1 ratio (VS basis). The substrate loading was maintained at 1.5 g VS/100 mL working volume of the serum bottle. Three serum bottles containing only the inoculum were used as a control to account for the volume of methane produced from the inoculum alone. All the experiments discussed in this study were conducted in triplicates. The serum bottle was sealed with a rubber stopper and was purged with nitrogen gas to strip off air from the headspace. Digestion was carried out in an incubator shaker (New Brunswick Scientific ExcellaTM E25, New Brunswick Scientific Co., Inc., USA) maintained at a mesophilic condition (37±1 °C) and 100 rpm. Daily biogas produced was collected in Tedlar bags (CEL Scientific Corporation, Cerritos, CA, USA), and the volume of the biogas was quantified by a mili-gas counter (Ritter US LLC., Hawthome, NY, USA). Gas volumes were normalized to standard temperature (273 K) and pressure (1 atm) and expressed as Nm^3. The composition of the biogas was analyzed using a micro-gas chromatograph (CP-4900 Micro-GC, Middelburg, Netherlands) equipped with thermal conductivity detector (TCD) and 10 m PPQ column (Agilent Technologies Inc., Wilmington DE, USA).

The digestion test was terminated after 42 days when the cumulative volume of the produced biogas reached a plateau. The bottle contents were mixed well, and the digestate sample was taken for TS and VS analyses. Digested residue was collected by sieving the digester contents through a #60 sieve (ASTM 250 μm, Thermo Fisher Scientific Inc., USA). The residue was washed four times with 500 mL of distilled water. After the fourth wash, the filtrate was visibly clear in color. The remaining fiber was analyzed for its TS, VS, and fiber composition (i.e., NDF, ADF, and ADL). The TS and VS content of the digested residue in the control serum bottle were also quantified, and fiber composition was analyzed as indicated above. The fiber contribution (with respect to NDF, ADF, and ADL) from the control serum bottle was subtracted from the samples of interest to quantify the digested residue of the substrate only (and not of the inoculum).

2.1.4 Analytical methods

TS and VS were determined as per Standard Methods (APPA, AWWA, WEF, 2005). The biomass was analyzed for NDF, ADF and ADL content by using the cell wall fractionation method according to ANKOM technology (ANKOM Technology, Macedon, NY, USA). For fiber compositional analysis, both the milled biomass (before digestion) and the washed digested residue were dried at 40 °C for two days. The samples were then milled using a standard feed processing mill (Thomas Model 2 Wiley® Mill, Thomas Scientific, Swedesboro, NJ, USA) equipped with a 1 mm screen. ADF and NDF extractions were determined using an ANKOM220 Fiber Analyzer (ANKOM Technology, NY, USA) with F57 filter bags (25 μm porosity). During NDF analysis, heat stable α-amylase was added in neutral-detergent solution to remove the any starch present in the biomass. Sodium sulfite was added to the solution and NDF was calculated as ash-free. The contents of the cell wall structural carbohydrates, namely hemicellulose and cellulose were calculated as the following differences: hemicellulose = NDF-ADF and cellulose = ADF-ADL (Hindrichsen et al., 2006; Butkute et al., 2014; Yue et al., 2010).

2.1.5 Statistical analyses

The experiment was designed using a split plot approach, where crop maturity stage was treated as the main plot effect, and size reduction was treated as sub-plot effect. All data were expressed as mean ± standard error (SE). Statistical significance was determined by analysis of variance (ANOVA) using Statistical Analysis System (SAS) software (SAS 9.2, SAS Institute Inc., Cary, NC, USA) with a threshold value (α) of 0.05.

2.2 Materials and Methods: Objective 2

2.2.1 Energy crops

The energy crop samples were collected on the island of Maui, Hawaii, USA, from field trial plots of the Biomass Research and Development Initiative (BRDI), a project designed to evaluate and identify high-yielding feedstocks for biofuel production. Details of the experimental sites, experimental design, planting and harvesting dates, and other crop management practices will be reported in the final report of a project [entitled "*conversion of high-yield tropical biomass into sustainable biofuels*" (Grant No. 2012-10006-19455, Project No.: HAW01512-G)] funded by U.S. Department of Agriculture's (USDA's) National Institute of Food and Agriculture (NIFA) though the Biomass Research and Development Initiative (BRDI), referred to as USDA, 2017 hereafter. For this study, crop samples of two C_4 perennial grasses, Energycane (*Saccharum hybrids*) and Napier grass (*Pennisetum purpureum*), were collected from three locations with elevations of 30 m, 305 m, and 915 m above mean sea level (msl), referred to as elevation hereafter. Within each crop type, two cultivars of each energy crop, Energycane (MOL

6136 and 77-9271) and Napier grass (Green and Purple), were evaluated across all three elevations. At the low (30 m) and middle (305 m) elevation sites, plots consisted of four 15 m long double rows. The rows within a double row were 0.9 m apart, and double rows were 1.8 m apart. At the high elevation (915 m) site, plots were three 4.6 m long double rows due to limited field area at the experimental site. Field trials were conducted in triplicates with three plots at each site for each crop.

Crop samples were collected from all three elevations for three years (from 2013 through 2015). Energycane was harvested every year in September, while Napier grass was harvested every six months in March and September of each year, hereafter referred to as March harvest and September harvest. Thus, throughout the study period, Energycane samples were collected three times, whereas Napier grass samples were collected six times. For the March 2013 harvest, Napier grass plots at 305 m elevation were destroyed by deer resulting in loss of that data. Thus, only four harvests of Napier grass from 2014 and 2015 were used for statistical analysis. Yield data were collected from a 9 m length of the middle double rows. For compositional and other analyses, a random sample of 10 stalks was taken. The selected stalks were separated into stems and leaves (blade and sheath). Energycane was further separated into four parts, dried and brown bottom leaves, bottom stem that had dried and brown leaves, green top leaves, and top stem that had green leaves. Since Napier grass was harvested every six months, it did not have distinct areas of dried/brown leaves and green leaves. Napier grass was shorter and greener throughout than Energycane. The separated plant parts were hand chopped to a length of 3 - 5 cm and dried at 65 °C. The dried biomass samples were shredded for initial size reduction followed by grinding to 2 mm size using a cutting mill (Retch

SM2000, Haan, Germany). The dried and ground biomass samples were packed in airtight zip-lock bags and stored at room temperature until further analysis. The energy crops, cultivars, elevations (locations), harvest years and seasons, and plant parts are presented in Table 2.1. At each site, automatic data loggers were used to record the daily weather data including air temperature, rainfall and solar radiation.

Table 2.1. Summary of energy crops, cultivars, plant parts, elevations and harvest dates

Energy crops	Cultivars	Plant parts	Elevations (m) (Locations)	Harvest dates
Napier grass	Green Purple	Stem Leaf	30 (718) 305 (410) 915 (Kula)	Mar., 2013, Sept., 2013 Mar., 2014, Sept., 2014 Mar., 2015, Sept., 2015
Energycane	MOL 6136 77-9271	Stem, Bottom Stem, Top Leaf, Bottom Leaf, Top	30 (718) 305 (410) 915 (Kula)	Sept., 2013 Sept., 2014 Sept., 2015

2.2.2 Fiber composition analysis

The dried and ground (2 mm) biomass samples were further milled to 1 mm size using a standard feed processing mill (Thomas Model 2 Wiley® Mill, Thomas Scientific, Swedesboro, NJ, USA) equipped with a 1 mm screen. The milled biomass was analyzed for its fiber composition including NDF, ADF, and ADL using the cell wall fractionation method according to ANKOM technology (ANKOM Technology, Macedon, NY, USA).

ADF and NDF extractions were done using an ANKOM200 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) with F57 filter bags (25 μm porosity). During NDF analysis, heat stable α-amylase was added in neutral-detergent solution to remove any starch content of the biomass. Sodium sulfite was added to the solution to remove the ash. ADL content was determined using the beaker extraction method (ANKOM Technology, Macedon, NY, USA). A mixed biomass sample was prepared by mixing leaf and stem samples of Energycane and Napier grass, and was used as a control to monitor process variation among the batches of fiber during washing/extracting biomass samples with respective detergents in the ANKOM fiber analyzer. Two bags of control sample were used in each batch of extraction. Average values for the NDF, ADF, and ADL content of control samples were derived for each batch, and correction factors were derived for NDF, ADF and ADL content and used to correct NDF, ADF and ADL values for each associated biomass sample. NDF, ADF and ADL values were also corrected for the residual ash content and are presented as ash free. Ash free extractives was determined as a biomass component other than NDF and ash, and was expressed as % of TS (i.e., 100% – ash% – NDF%). The cell wall structural carbohydrates, hemicellulose and cellulose, were estimated by difference: hemicellulose = NDF - ADF and cellulose = ADF - ADL (Hindrichsen et al., 2006; Yue et al., 2010; Waramit et al., 2011; Butkutė et al., 2014; Na et al., 2014b). Field plots were used as replicates (n = 3) and lab analyses for determining NDF, ADF and ADL content were not replicated.

2.2.3 Statistical analyses

Data were analyzed using a split-split-split-split plot model using JMP Pro statistical software (v.12, SAS Institute Inc., USA), with elevation as main plot effect, cultivar as subplot effect, plant part was used as sub-subplot effect and harvest year, or harvest season for Napier grass, as a repeated sub-sub-subplot effect. Total aboveground biomass composition was derived as the weighted average of plant parts [leaves and stems for Napier grass and leaves (bottom and top) and stems (bottom and top) for Energycane] and compared for locations (as indicated by different elevations), cultivars and harvests. Test of significance was done on main effects and their interactions. Means were compared using Tukey's test. Unless stated otherwise, all the means reported are least square means and differences are considered significant if $p \leq 0.05$. All percentages are on a dry matter basis unless stated otherwise. Since the Napier grass data from elevation 305 m for March 2013 harvest were missing due to deer damage in the experimental plots, data from 2014 and 2015 (March and September) harvests only were used in the analysis. Pearson's correlation coefficients were calculated to determine the correlation between the biomass yield and biomass composition, and were considered significant if $p \leq 0.05$.

2.3 Materials and Methods: Objective 3

2.3.1 Substrate

The dried and milled biomass (1 mm in size) samples of Energycane and Napier grass harvested in 2015 across three elevations were used for digestibility study. The biomass samples collection, preparation and characterization were described in detail in the subsections 4.2.1 and 4.2.2.

2.3.2 Inoculum

The inoculum was taken from the mother reactor maintained in the State Institute of Agricultural Engineering and Bioenergy at University of Hohenheim, Stuttgart, Germany. The mother reactors were fed mainly with cattle manure and kept at a mesophilic temperature. To minimize the contribution of inoculum to methane yield, the contents of the mother reactor were sieved using a kitchen strainer and the filtrate was used as an inoculum. The TS and VS contents of the inoculum were 6.24 ± 0.25% and 60.97 ± 0.52% of TS, respectively. For each batch test, fresh inoculum was taken out from the mother reactor and sieved just prior to the start of the digestion test to avoid storage.

2.3.3 Digestion test

Digestion study was conducted following the Hohenheim Biogas Yield Test (HBT) (Mittweg et al., 2012). The HBT used a series of 100 mL glass syringe as a digester where 500 mg of dried and milled (1 mm size) biomass was digested using 30 gm (wet weight) of active anaerobic inoculum at mesophilic condition (37 ± 0.5 °C) for 35 days. Two standard biomass samples (i.e., hay and concentrated feed) with known biomass composition and biomethane production potential were used as control to check the quality of inoculum as well as to account for the variation among the batch tests due to inoculum activity and other analytical variation. Three syringes containing only the inoculum were used as a control to account for the volume of biogas and methane produced from the inoculum alone. Correction factor was obtained for each batch of digestibility test based on methane yield data of the standards and was used for correcting the methane yield obtained in each batch test. Digestion tests were conducted in triplicates. The volume of biogas and methane yield was monitored over the digestion period. The volume of the biogas was determined by reading the filling level of the glass syringe. The methane content in the biogas was determined using an infrared-spectrometric methane-sensor (Advanced Gasmitter, Pronova Analysetechnik, Berlin, Germany). The analyzer was calibrated with a calibration gas (i.e., 60% CH_4), pre- and post-measurement. The measured volumes of biogas and methane were normalized to standard conditions (273 K and 1 atm). Standard incubation time for HBT was 35 days. However, digestibility test of selected samples was conducted for the incubation time of 90 days to test the effect of incubation time on methane production potential.

2.3.4 Statistical analyses

The data was analyzed using a split-split-split-plot model using JMP Pro statistical software (v.12, SAS Institute Inc., USA), where elevation was treated as the main plot effect, cultivar as sub-plot effect, plant part as sub-sub-plot effect and harvest season (Napier grass) as sub-sub-sub-plot effect. Pearson's correlation coefficients were calculated to determine the correlation between the biomass composition, methane yield and the interrelation among chemical constituents.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Results and Discussion: Objective 1

3.1.1 Biomass composition

With respect to maturity, the TS and VS contents of the biomass increased significantly ($\alpha = 0.05$) as summarized in Table 3.1. The increase in the TS and VS contents and decrease in ash content for Napier grass were also reported by Takara (2012) and for other grass species by Butuke et al. (2014). Higher VS content (and less ash) as a result of maturation could be due to (i) the translocation of nutrient components to underground sections (e.g., the root) and (ii) leaching as a result of natural weathering processes (e.g., rain).

The total lignocellulose (i.e., NDF) content (on a % TS basis) of Napier grass showed an increasing trend (positive correlation) with maturity. Compared to biomass harvested at 2 months old, the lignocellulose content [namely, lignin (ADL), hemicellulose, and cellulose] increased by 10% for biomass harvested at 8 months of age. There was no significant difference ($\alpha = 0.05$) in the total lignocellulose content between the biomass harvested at 6 and 8 months of age. The cellulose and lignin (ADL) content of the biomass increased significantly ($\alpha = 0.05$) with crop maturity. In particular, the cellulose and lignin (ADL) content increased by 10% and 82%, respectively, for the 8 month biomass compared to the 2 month old crop. No significant ($\alpha = 0.05$) difference in the lignin (ADL) content of biomass harvested at 6 and 8 months was observed. Interestingly, the hemicellulose fraction of Napier grass was found to decrease as the plant aged. The hemicellulose content of the crop harvested at 2 months of age was significantly ($\alpha = 0.05$) higher than the hemicellulose content of the 4, 6 and 8 months old biomass. Though the hemicellulose content was not significantly ($\alpha = 0.05$) different among the 4, 6 and 8 months crops, there was a decreasing trend seen in the hemicellulose content of aging biomass. The NDF, ADF, cellulose, hemicellulose and lignin (ADL) content of the ratooned Napier grass are summarized in Table 3.1.

3.1.2 Specific methane yield

3.1.2.1 Effect of crop maturity

The specific methane yield of biomass harvested under different stages of maturation and grinding regimes is summarized in Table 3.2. There was a significant difference ($\alpha = 0.05$) in the specific methane yield among crops harvested at varying ages. More specifically, the specific methane yield decreased significantly ($\alpha = 0.05$) with increasing crop maturity. Feedstock harvested at 2 months old resulted in the highest specific methane yield compared to crops harvested at later stages of maturity. This increase in the specific methane yield for 2 month old biomass was 16%, 67%, and 110% higher than the biomass harvested at 4, 6 and 8 months of age, respectively.

3.1.2.2 Effect of size reduction

The size reduction (grinding) of biomass also exhibited significant effects ($\alpha = 0.05$) on the digestibility and ultimate specific methane yield. For all stages of biomass maturity, it was found that biomass passed through the 6 mm sieve resulted in a significantly ($\alpha = 0.05$) higher specific methane yield compared to the biomass passed through 10 and 20 mm sieves. An inverse relationship of the specific methane yield with sieve size was observed for the biomass harvested at 2, 4, 6 and 8 months age.

Table 3.1. Changes in the composition of ratooned Napier grass with respect to maturity (n = 3) [numbers followed by similar letter in the same column are not significantly (α = 0.05) different]

Maturity (months)	Total solids (%)	Volatile solids (% TS)	NDF (% TS)	ADF (% TS)	Lignin (ADL) (% TS)	Hemicellulose (% TS)	Cellulose (% TS)
2	16.06. ± 0.11[a]	81.39 ± 0.33[a]	66.13 ± 0.09[a]	45.18 ± 0.11[a]	6.24 ± 0.07[a]	20.95 ± 0.20[a]	39.00 ± 0.08[a]
4	23.22 ± 0.18[b]	83.91 ± 0.16[b]	69.10 ± 0.25[b]	50.07 ± 0.25[b]	8.72 ± 0.03[b]	19.03 ± 0.15[b]	41.35 ± 0.22[b]
6	30.29 ± 0.30[c]	87.14 ± 0.16[c]	73.18 ± 0.51[c]	53.45 ± 0.41[c]	11.07 ± 0.24[c]	19.73 ± 0.43[b]	42.38 ± 0.22[c]
8	37.76 ± 0.53[d]	91.60 ± 0.28[d]	73.02 ± 0.44[c]	54.35 ± 0.08[d]	11.34 ± 0.14[c]	18.67 ± 0.48[b]	43.01 ± 0.21[d]

Table 3.2. Specific methane yield of the ratooned Napier grass at different stages of maturity

Maturity (months)	Biomass size (mm)	Specific methane yield (Nm^3 (kg VS_{added})$^{-1}$)
2	6	0.236 ± 0.0002
	10	0.218 ± 0.0008
	20	0.203 ± 0.0005
Mean		**0.219 ± 0.0049**
4	6	0.217 ± 0.0013
	10	0.181 ± 0.0030
	20	0.168 ± 0.0005
Mean		**0.189 ± 0.0073**
6	6	0.150 ± 0.0008
	10	0.123 ± 0.0030
	20	0.121 ± 0.0014
Mean		**0.131 ± 0.0047**
8	6	0.112 ± 0.0021
	10	0.103 ± 0.0010
	20	0.98 ± 0.0006
Mean		**0.104 ± 0.0023**

The specific methane yield obtained in this study was within the range of the specific methane yield reported by McEnirey and O'Kiely (2013) for five different grass species (perennial ryegrass, Italian ryegrass, Timothy, Cocksfoot, and Tall fescue) at three stages (i.e., stem elongation, reproductive development, and anthesis) of growth. The specific methane yield reported by the authors varied from 0.207 - 0.263 Nm^3 methane (kg VS_{added})$^{-1}$. Masse et al. (2010) also reported specific methane yields ranging from 0.191 - 0.309 Nm^3 methane (kg VS_{added})$^{-1}$ for switchgrass silage harvested at different stages of development. The specific methane yield obtained in the present experiment, however, is comparatively less than the specific methane yields mentioned for other grass species reported by Kaiser and Gronauer (2007) [0.198 - 0.345 Nm^3 methane (kg VS_{added})$^{-1}$] and Seppalla et al. (2009) [0.250 - 0.390 Nm^3 methane (kg VS_{added})$^{-1}$]. This could be due to the difference in the biomass composition as well as the larger particle sizes as the biomass samples in this experiment were not finely milled to a powder as in other studies.

There were significant ($\alpha = 0.05$) interactive effects with respect to size reduction and crop maturity on the overall specific methane yield. It was observed that biomass harvested at the earliest stages of maturity (2 month), and passed through the smallest sieve (6 mm), resulted in the highest specific methane yields (Figure 3.1) compared to biomass harvested at later stages of maturity (4, 6 and 8 months) and passed through larger sieves (10 and 20 mm). It was also observed that the effects of size reduction on specific methane yield were more distinct for the biomass harvested at younger ages. For the biomass harvested in the later stages of maturity, the effect of size reduction was not as evident as biomass harvested at younger age. From Figure 3.1, it can be seen that there was not much difference in the specific methane yields for the biomass harvested at the later stages of maturity (e.g., 6 and 8 months) and passed through larger (e.g., 10 and 20 mm) sieves.

The digestibility of lignocellulose (described later in the section 3.1.3) was positively correlated with the specific methane yield (Figure 3.2). The changes in the lignocellulose digestibility with respect to maturity are likely related to differences in plant composition with respect to maturity. The hemicellulose content of the biomass, which was observed to be inversely proportional to maturity, correlated positively with methane yields. In contrast, the cellulose and lignin (ADL) content, which increased significantly ($\alpha = 0.05$) with respect to crop maturity, was found to have a negative correlation with the overall methane yield. Differences in the digestibility of the hemicellulose and cellulose (to produce methane) can be explained based on the structural differences of these biomass components. A lower degree of polymerization (100 - 200 units) and relatively amorphous structure makes hemicellulose readily biodegradable compared to cellulose (Perez et al., 2002; Li et al., 2010). The degree of polymerization and crystallinity of cellulose has been shown to increase with maturity due to the increased compactness of the hydrogen bonds inherent in the structure (Agrawal, 2007; Brown, 2003; Cherubini, 2010; Kumar et al., 2008).

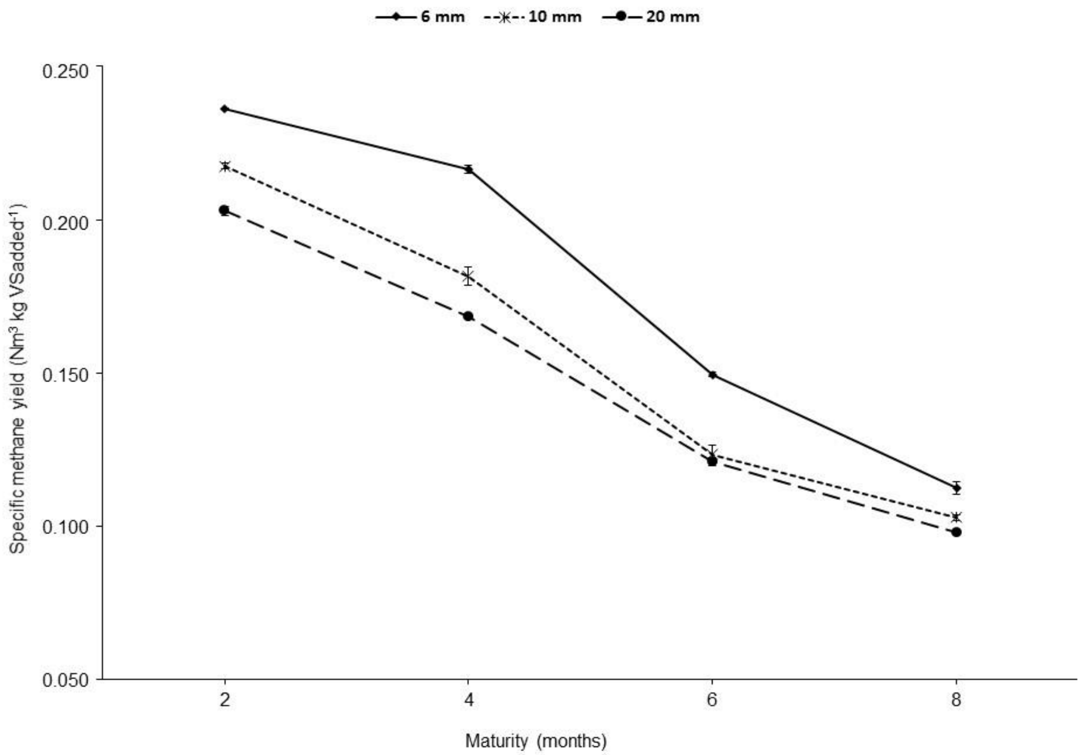

Figure 3.1. The effect of crop maturity and size reduction (6, 10, and 20 mm) on the specific methane yield in 42 day batch digestion test

Several AD studies on different grass species have shown a positive correlation between the lignocellulose content and plant maturity, and negative correlation of lignocellulose content and the specific methane yields (McEniry and O'Kiely, 2013; Kaparaju et al., 2002; Seppala et al., 2009). The changes in the composition of biomass with different harvest times (maturity) found in this study agrees with the results of other energy crops digestion studies including (but not limited to) Butkute et al., (2014), McEnirey and O'Kiely (2013), and Rezaeifard (2010). Early harvests of Napier grass in subtropical regions may facilitate better specific methane yield with minimal preprocessing.

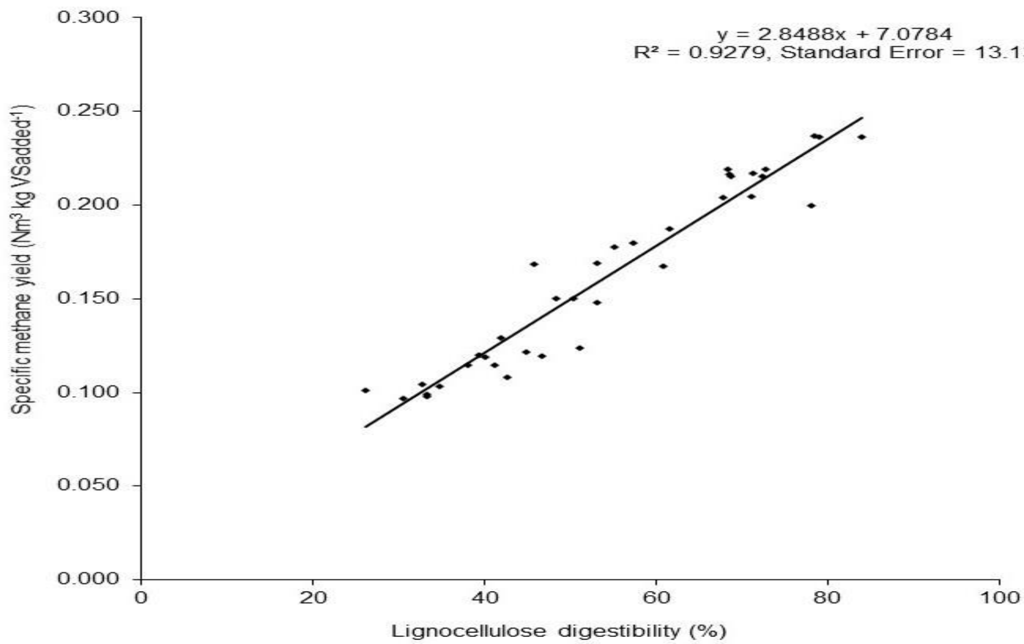

Figure 3.2. Linear regression of the specific methane yield with lignocellulose digestibility in 42 day batch digestion test

Table 3.3. The digestibility of lignocellulose (i.e., % solubilized and/or consumed) after anaerobic digestion (n=3) (numbers followed by similar letter in the same column are not significantly (α=0.05) different)

Maturity (months)	NDF	ADF	Hemicellulose	Cellulose
2	74.10 ± 1.97^a	69.32 ± 2.34^a	84.40 ± 1.66^a	78.74 ± 1.92^a
4	60.86 ± 3.05^b	58.38 ± 3.07^b	67.38 ± 3.42^b	66.23 ± 3.21^b
6	46.22 ± 1.65^c	41.83 ± 1.79^c	58.13 ± 1.61^c	46.80 ± 1.79^c
8	34.72 ± 1.72^d	33.50 ± 1.69^d	38.25 ± 1.95^d	37.11 ± 1.85^d

3.1.3 Lignocellulose digestibility

The digestibility of lignocellulose during AD varied significantly ($\alpha = 0.05$) among the biomass harvested at different ages. Nearly 74.10 ± 1.97% of lignocellulose was digested during the digestion of 2 month old biomass, whereas the digestibilities for 4, 6 and 8 months old biomass were 60.86 ± 3.05%, 46.22 ± 1.65%, and 34.72 ± 1.72%, respectively. The digestibilities of fiber components are summarized in the Table 3.3. The digestibility of the hemicellulose varied from 84.40 ± 1.66% (2 month) to 38.25 ± 1.95% (8 month). Similarly, cellulose digestibility decreased from 78.74 ± 1.92% (2 month old biomass) to 37.11 ± 1.85% (8 month old biomass) (Table 3.3).

Table 3.4. Fiber composition of digested residue (n=3) [numbers followed by similar letter in the same column are not significantly (α=0.05) different] (Unit: % dry weight)

Maturity (months)	NDF	ADF	Lignin (ADL)	Hemicellulose	Cellulose
2	69.73 ± 0.94^a	56.48 ± 0.63^a	23.06 ± 0.86^a	13.26 ± 1.04^a	33.42 ± 0.85^a
4	76.21 ± 1.56^b	58.82 ± 1.03^b	19.82 ± 0.99^b	17.39 ± 1.10^b	39.01 ± 1.66^b
6	83.49 ± 0.71^c	65.96 ± 0.56^c	18.15 ± 0.44^c	17.53 ± 0.44^b	47.81 ± 0.64^c
8	88.14 ± 0.45^d	66.84 ± 0.23^c	16.85 ± 0.35^d	21.30 ± 0.32^c	49.99 ± 0.42^c

The fiber composition of the digested residue is summarized in the Table 3.4. Compared to the control (feedstock before digestion), the digested residue contained higher amounts of lignocellulose (% TS). The lignocellulose content in the digested residue appeared to increase by 5% for the crop harvested at 2 months of age while there appeared to be a 10%, 14% and 20% increase in the lignocellulose content (% TS) in the digested residue of 4, 6 and 8 months biomass, respectively. The observed increase, however, was misleading. Since it is impossible for lignocellulose to increase (following the law of conservation of mass), the values were likely biased high because of microbes consuming non-structural (and non-carbohydrate) components of the plant. The composition was calculated on a % TS basis, therefore, all the remaining plant constituents appeared higher than in the non-digested biomass (biomass before digestion). Compared to the biomass (before digestion), the lignin (ADL) content was higher in the digested residue of all aged biomass (i.e., 2, 4, 6 and 8 month old biomass). This observation can be explained by the comparatively higher recalcitrance of lignin to biological degradation than that of hemicellulose and cellulose. The hemicellulose content, however, was lower (compared to the biomass before digestion) in the digested residue of the biomass harvested at 2, 4 and 6 months of maturity, and only the residue of the 8 month old biomass showed higher concentration of hemicellulose. Similarly, the cellulose content was lower in the digested residue of the 2 and 4 month old biomass, whereas higher cellulose content was observed in the digested residue of the 6 and 8 months matured biomass.

The significant reduction in cellulose content of the digested residue of the younger biomass may be attributed to less lignin (which suggests that cellulose is more readily accessible for microbial attack) thereby making cellulose more amenable to biological hydrolysis than the highly crystallized cellulose in the biomass harvested in the later stage of maturity (Agrawal, 2007; Brown, 2003; Cherubini, 2010). Additionally, the lower concentration of lignin (ADL) in the younger harvested biomass suggests less recalcitrance to the biological breakdown of hemicellulose and cellulose into the simple sugars, and finally to the end product, methane. The specific methane yield from the biomass harvested at the later stages of maturity could mainly be attributed to the non-fiber components (i.e., other than NDF) of the biomass such as water-soluble carbohydrates and crude proteins. Though the Napier grass is typically high in lignocellulose (i.e., NDF), the specific methane yield obtained in this study was low. The results indicate, however, that there is potential in improving the biomass conversion to the specific methane by harvesting Napier grass at specific stage of maturity and applying appropriate size reduction strategies.

3.2 Results and Discussion: Objective 2

The average monthly weather data for the three elevations for the years 2012 through 2015 are summarized in Figure 3.3. There was significant difference in rainfall ($p = 0.0465$), temperature ($p < .0001$) and solar radiation ($p < .0001$) across the elevations. Average monthly rainfall was highest at the highest elevation (48.43 ± 5.73 mm) followed by the middle and the lowest elevation, 34.57 ± 5.73 mm and 28.66 ± 5.73 mm, respectively. Average monthly rainfall, however, was not significantly different between the highest and the middle elevation, and between the middle and the lowest elevation. The lowest elevation had the highest average monthly temperature (23.24 ± 0.24 ˚C) and solar radiation (20.43 ± 0.43 MJ m^{-2} day^{-1}), followed by the middle (22.34 ± 0.24 ˚C and 19.32 ± 0.43 MJ m^{-2} day^{-1} of temperature and solar radiation, respectively) and the highest elevation (17.73 ± 0.24 ˚C and 15.55 ± 0.43 MJ m^{-2} day^{-1} of temperature and solar radiation, respectively). Average monthly solar radiation was not significantly different between the lowest and the middle elevation.

The biomass yields of both crops across harvest years and elevations will be discussed in detail by USDA, (2017). However, a summary of biomass yield of different plant parts and whole crop for both crop types in this study is presented in Figure 3.4 and Figure 3.5, respectively.

3.2.1 Biomass composition of different plant parts

The NDF, ADF, lignin (ADL), cellulose, hemicellulose, ash and ash free extractive content of the plant parts of Energycane and Napier grass are summarized in Table 3.5 (Energycane) and Table 3.6 (Napier grass). As shown in the tables, in most cases, ash, ash free extractive, NDF, ADF, cellulose, hemicellulose and lignin (ADL) content of the biomass varied significantly by plant parts. Plant parts of both energy crops interacted significantly with elevation, harvest year and cultivar for most of the parameters examined. Least square mean values for the main effect (plant part) followed by the minimum and maximum values of the least square means for the highest level of interaction (plant part by elevation by cultivar by harvest year/season) are presented in the tables (Tables 3.5 and 3.6). The least square means of highest level of interactions for each parameter studied are summarized in the appendix B.

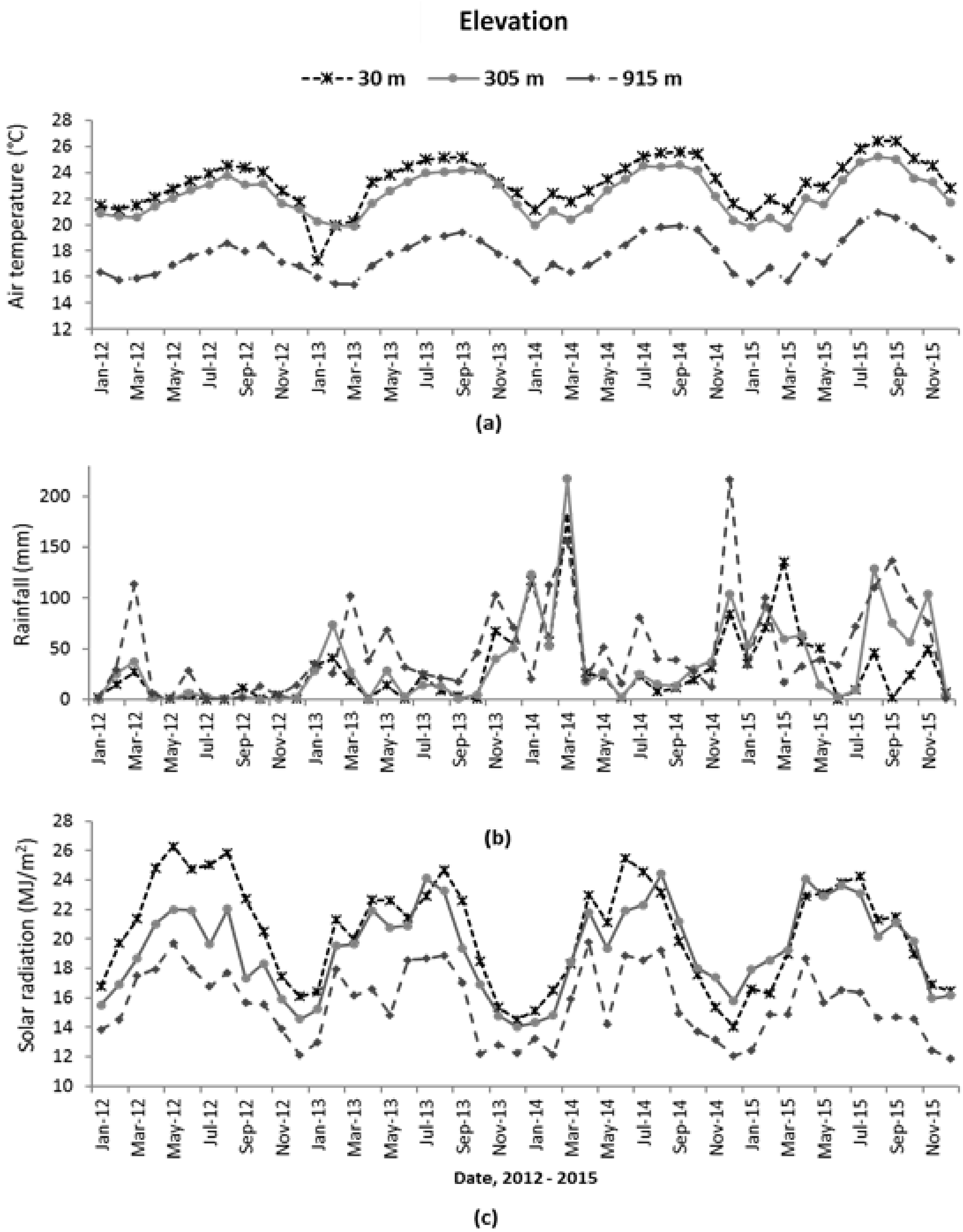

Figure 3.3. Average monthly weather data; (a) air temperature (˚C), (b) rainfall (mm), and (c) solar radiation (MJ m^{-2} day^{-1}); across three elevations (30 m, 305 m, and 915 m) for 2012 through 2015.

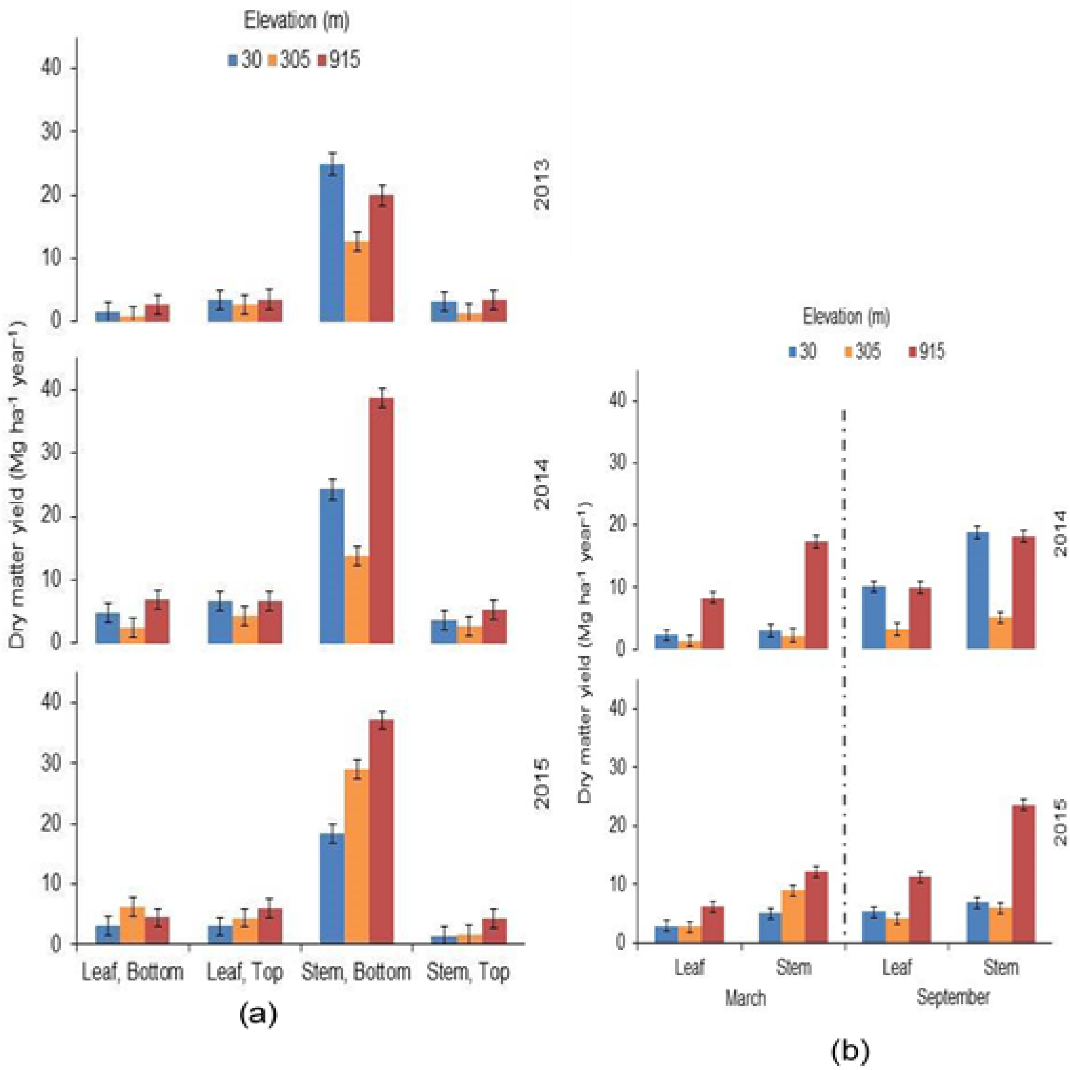

Figure 3.4. Dry matter yield of different plant parts of (a) Energycane and (b) Napier grass across the elevations (30 m, 305 m, and 915 m) and harvest years (2013 through 2015)/seasons (March and September).

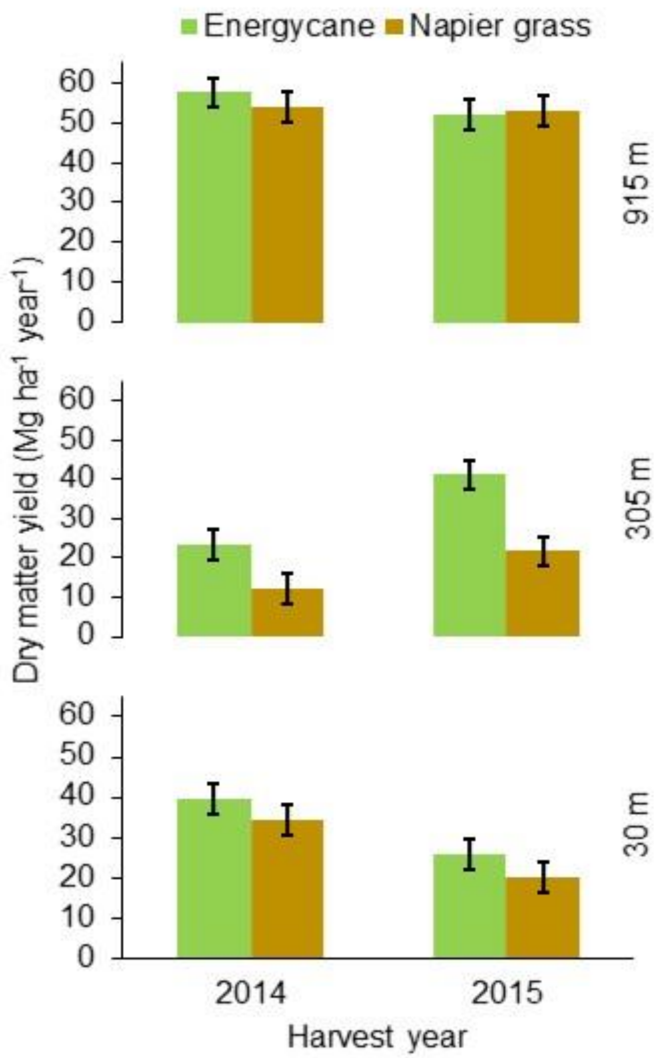

Figure 3.5. Dry matter yield of Energycane and Napier grass across the elevations (30 m, 305 m, and 915 m) and harvest years (2014 and 2015).

As summarized in the Table 3.5, the bottom leaves of the Energycane had the highest ash, NDF, ADF and cellulose content followed by the top leaves, top stems and bottom stems. The hemicellulose content, however, was highest in the top leaves followed by the bottom leaves, top stems, and was lowest in the bottom stems. Whereas the bottom leaves had the highest lignin (ADL) content followed by the bottom stems, top leaves and top stems.

When compared between leaves and stems, Energycane leaves had significantly higher ash ($p <.0001$), NDF ($p <.0001$), ADF ($p <.0001$), cellulose ($p <.0001$) and hemicellulose ($p <.0001$) content than stems, while no significant ($p = 0.0768$) difference was found in lignin (ADL) content between leaves and stems. Energycane leaves had 125.38%, 37.63%, 21.06%, 26.18% and 73.58% higher ash, NDF, ADF, cellulose and hemicellulose content, respectively, than stems.

Unlike Energycane, Napier grass stems had significantly higher NDF, ADF, cellulose and lignin (ADL) content than leaves, while leaves were significantly higher in hemicellulose and ash free extractive content than stems (Table 3.6). Compared to the leaves, Napier grass stems had 12.49%, 37.05%, 25.77% and 126.19% higher NDF, ADF, cellulose and lignin (ADL) content, respectively, while leaves had 32.06% and 48.10% higher hemicellulose and ash free extractives content than stems. However, ash content was not significantly different between leaves and stems of Napier grass.

Plant part by elevation interaction was significant ($p <.0001$) for ash, ash free extractives, NDF, ADF, hemicellulose, cellulose and lignin (ADL) content in Energycane, while plant part by harvest year interaction was significant for ash ($p <.0001$), NDF ($p = 0.0017$), ADF ($p = 0.0046$) and lignin (ADL) ($p <.0001$) content. Similarly, plant part by cultivar interaction was significant for ash free extractives ($p = 0.0124$), NDF ($p <.0001$), ADF ($p = 0.0015$), hemicellulose ($p= 0.0090$), cellulose ($p = 0.0001$), and lignin (ADL) ($p = 0.0328$). In Napier grass, plant part by elevation interaction was significant for ash ($p <.0001$), ash free extractives ($p <.0001$), NDF (p

<.0001), ADF (p = 0.0001), hemicellulose (p = 0.0034) and cellulose (p <.0001) content. Similarly, plant part by harvest season was significant for NDF (p = 0.0066), ADF (p = 0.0007), cellulose (p = 0.0233) and lignin (ADL) (p <.0001) content, but plant part by harvest year interaction was significant only for lignin (ADL) (p = 0.0073) content.

As shown in Table 3.7, dry matter yields of plant parts were differently correlated with the respective composition of plant parts for Energycane and Napier grass. In Energycane, dry matter yields of the bottom and top stems were negatively correlated with ash, NDF, ADF, hemicellulose, cellulose and lignin (ADL) content in the respective plant parts, while dry matter yield of the bottom leaves was positively correlated with NDF, ADF, cellulose and lignin (ADL) content. The dry matter yield of the top leaves, however, had a significant negative correlation with hemicellulose content. Similarly, dry matter yield of Napier grass leaves had negative correlations with NDF and hemicellulose content and positive correlations with ash and lignin (ADL) content in leaves. The stem dry matter yield, however, had negative correlations with ash and hemicellulose content, and positive correlations with NDF, ADF and cellulose content in stems. As shown in Figure 3.4, the dry matter yields of various plant parts of Energycane and Napier grass varied with elevations and years (and seasons in Napier grass). Thus, the variation in dry matter yields of plant parts across elevations and harvest years (seasons in Napier grass), and different correlations of dry matter yields of plant parts with their composition could have resulted in the significant interaction of plant part composition with elevation and harvest years (and seasons in Napier grass).

Table 3.5. Least square means of composition of different plant parts of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry weight)

Factors	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
Leaf, Bottom	9.32 ± 0.19[a] (5.00 - 13.71)	18.61 ± 0.41[a] (15.58 - 22.07)	72.07 ± 0.36[a] (65.34 - 79.40)	45.62 ± 0.35[a] (39.85 - 50.16)	26.45 ± 0.22[a] (21.94 - 29.34)	39.08 ± 0.29[a] (34.42 - 42.47)	6.54 ± 0.10[a] (5.43 - 7.85)
Leaf, Top	8.88 ± 0.20[a] (4.04 - 12.97)	21.70 ± 0.42[b] (16.19 - 25.67)	69.42 ± 0.37[b] (65.58 - 72.91)	40.26 ± 0.35[b] (38.04 - 42.95)	29.16 ± 0.22[b] (26.83 - 33.20)	34.93 ± 0.29[b] (32.98 - 36.68)	5.32 ± 0.10[b] (4.70 - 6.27)
Stem, Bottom	3.50 ± 0.20[b] (1.15 - 7.64)	46.56 ± 0.43[c] (35.49 - 57.28)	49.90 ± 0.38[c] (40.21 - 60.35)	34.65 ± 0.36[c] (26.84 - 43.35)	15.27 ± 0.23[c] (11.76 - 17.17)	28.47 ± 0.30[c] (22.58 - 32.88)	6.18 ± 0.10[c] (4.11 - 8.20)
Stem, Top	8.01 ± 0.19[c] (3.67 - 11.29)	28.83 ± 0.41[c] (23.43 - 35.31)	63.16 ± 0.36[d] (59.11 - 66.83)	39.19 ± 0.35[d] (35.50 - 42.27)	23.97 ± 0.22[d] (21.07 - 26.79)	34.14 ± 0.29[d] (32.80 - 36.62)	5.05 ± 0.10[b] (4.02 - 6.52)
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

*Least square mean values ± standard error (minimum value – maximum value) (n = 54) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table 3.6. Least square means of composition of different plant parts of the Napier grass cultivars collected across the three elevations and the two harvest years (four harvest seasons) (Unit: % dry matter)

Factor	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
Leaf	8.12 ± 0.33[a] (3.43 - 15.30)	26.48 ± 0.36[a] (19.94 - 31.47)	65.40 ± 0.34[a] (62.31 - 71.39)	39.07 ± 0.30[a] (35.33 - 42.88)	26.33 ± 0.17[a] (23.84 - 29.71)	34.71 ± 0.21[a] (31.30 - 38.48)	4.36 ± 0.12[a] (3.66 - 5.21)
Stem	8.63 ± 0.33[a] (2.74 - 15.96)	17.88 ± 0.36[b] (10.20 - 24.12)	73.45 ± 0.34[b] (67.03 - 79.58)	53.19 ± 0.30[b] (46.36 - 61.74)	20.25 ± 0.17[b] (16.66 - 22.69)	43.51 ± 0.21[b] (39.41 - 49.41)	9.68 ± 0.12[b] (6.95 - 12.50)
P value	0.3549	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

*Least square mean values ± standard error (minimum value – maximum value) (n = 36) followed by a different letter within a column are significantly different at the P ≤ 0.05 level

In general, the lower fiber (lignocellulose; NDF) content in the bottom stems of Energycane is due to the substantial proportion of nonstructural components (soluble solids including soluble sugars) present in the Energycane bottom stems. The brix value in bottom stems of Energycane varied from 10 - 20% (data not presented here). Studies have shown an accumulation of substantial nonstructural components (soluble total solids) in the stems of mature Energycane (Bischoff et al., 2008; Fedenko et al., 2013; Na et al., 2014a). Thus, the lower fiber content, including NDF, ADF, cellulose and lignin (ADL), in stems than in leaves of Energycane and in both stems and leaves of Napier grass is due to the higher proportion of nonstructural components present in the Energycane stems.

When cellulose, hemicellulose and lignin (ADL) content was expressed in terms of relative concentration in fiber content, as a percentage of NDF content, the bottom stems had the highest cellulose (57.05 % NDF) and lignin (ADL) (12.38 % NDF) content compared to the top stems [cellulose = 54.04 % NDF; lignin (ADL) = 7.99 % NDF], bottom leaves [cellulose = 54.22 % NDF; lignin (ADL) = 9.07 % NDF] and top leaves [cellulose = 50.32 %; lignin (ADL) = 7.66 % NDF]. Hemicellulose content, however, was highest in the top leaves (42.05 % NDF) followed by top stems (37.95 % NDF), bottom leaves (36.70 % NDF), and bottom stems (30.60 % NDF).

da Costa et al. (2014) reported a significant difference in cell wall composition between actively growing and senescent stems of Miscanthus, which was attributed to the lower proportion of secondary cell walls in actively growing stems compared to the stem at senescence. Thus, the difference in the composition of the top and bottom stems or the top and bottom leaves observed in this study could be due to the difference in the maturity of leaf and stem tissues between top and bottom parts of the plant. Since the top leaves and stems are still green and are actively growing, the top leaves and stems could have a lower proportion of secondary cell walls compared to the bottom leaves and stems.

Several studies have reported significant differences in the composition of leaves and stems of several crop species, such as Miscanthus (Hodgson et al., 2010; Wahid et al., 2015; Weijde et al., 2016), Napier grass (Ansah et al., 2010; Khairani et al., 2013), sorghum (Murray et al., 2008) and reed canary grass (Kandel et al., 2013). Generally, lower NDF and cellulose, and higher hemicellulose content was reported in leaves than stems of Miscanthus (Hodgson et al., 2010; Wahid et al., 2015; Weijde et al., 2016). Similarly, higher lignin content in the stems than leaves was reported in varieties of lignocellulosic crops, such as Switchgrass (Mann et al., 2009; Shen et al., 2009), Miscanthus (Hodgson et al., 2010; Wahid et al., 2015), Napier grass (Ansah et al., 2010), sorghum (Murray et al., 2008) and reed canary grass (Kandel et al., 2013).

Similarly to the findings of this study, higher NDF, ADF, cellulose and lignin (ADL) content in stems than in leaves, and higher hemicellulose content in leaves than in stems of various cultivars of Napier grass was reported by Ansah et al. (2010). Khairani et al., (2013) also found higher cellulose in stems than leaves and higher hemicellulose content in leaves than stems of Napier grass. The NDF, ADF, cellulose, hemicellulose and lignin (ADL) content in leaves and stems of Napier grass found in this study were in close agreement with other studies of various Napier grass accessions and varieties (Ansah et al., 2010; Kebede et al., 2016).

Table 3.7. Pearson correlation between dry matter yield and composition of Energycane and Napier grass plant parts (plant part dry matter yield as variable and plant part composition as by variable)

Variables/ by variables (%TS)	Dry matter yield, Energycane								Dry matter yield, Napier grass							
	Bottom stems (n = 50)		Top stems (n = 54)		Bottom leaves (n = 54)		Top leaves (n = 53)		Stems total (n = 50)		Leaves total (n = 53)		Stems (n = 35)		Leaves (n = 36)	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
Ash	-0.3490	0.0130	-0.2243	0.1030	0.0008	0.9952	0.0299	0.8315	-0.3441	0.0144	0.1443	0.3027	-0.7763	<.0001	0.6949	<.0001
NDF	-0.3692	0.0083	-0.5182	<.0001	0.4053	0.0024	-0.1970	0.1575	-0.4072	0.0033	0.1462	0.2961	0.6284	0.0001	-0.3515	0.0355
ADF	-0.2669	0.0610	-0.2061	0.1348	0.4545	0.0006	0.1302	0.3529	-0.2996	0.0345	0.4607	0.0005	0.8022	<.0001	0.1117	0.5167
Lignin (ADL)	-0.1794	0.2125	-0.4445	0.0008	0.2861	0.0360	0.1697	0.2245	-0.2304	0.1075	0.3255	0.0174	0.2732	0.1123	0.2937	0.0821
Hemicellulose	-0.3772	0.0069	-0.5623	<.0001	0.0784	0.5732	-0.3622	0.0077	-0.4163	0.0026	-0.2886	0.0361	-0.4456	0.0073	-0.5347	0.0008
Cellulose	-0.2866	0.0436	-0.0845	0.5433	0.4137	0.0019	0.0777	0.5804	-0.3082	0.0295	0.4461	0.0008	0.8667	<.0001	0.0380	0.8260
Ash free extractives	0.4103	0.0031	0.4913	0.0002	-0.5854	<.0001	0.1272	0.3643	0.4355	0.0016	-0.2925	0.0336	0.2897	0.0913	-0.6085	<.0001

*r = correlation coefficient

Due to less fiber and substantial nonstructural solid (including soluble sugar) content, the bottom stems of Energycane could be an excellent feedstock for biochemical conversion. However, the fiber component (NDF), which contributes about 50% of total biomass, could be recalcitrant to biological conversion due to a relatively higher proportion of lignin (ADL) in the fiber (NDF). On the other hand, physical and chemical pretreatments to deconstruct the structural components of the stems for enzymatic saccharification may degrade the nonstructural carbohydrates (soluble sugars) with the concurrent formation of inhibitory compounds, such as hydroxymethylfurfural (HMF), which could ultimately inhibit the downstream biochemical conversion (enzymatic saccharification and fermentation). Thus, green processing as discussed in Takara and Khanal (2011) or an anaerobic digestion-based biorefinery (Surendra et al., 2015) could be used for an efficient conversion of Energycane bottom stems into biofuels and biobased products. In green processing, feedstock is usually pressed to extract the nonstructural carbohydrates, moisture and soluble nutrients. The extracted juice is used for biofuel and biobased product generation, while the fibrous residue is used either for cellulosic biofuel production following biomass pretreament or for thermochemical conversion (pyrolysis, gasification, hydrothermal liquifaction, torrefaction etc.). Similarly, in an anaerobic digestion-based biorefinery, the nonstructural carbohydrates and easily digestible component such as hemicellulose is converted to organic acids or biogas using naturally occurring mixed anaerobic microbial consortia, and the undigested fiber residue (digestate) can be used either for cellulosic biofuel (or biobased product) generation or themochemical conversion as discussed in Surendra et al. (2015) and Sawatdeenarunat et al. (2017).

Due to comparatively low lignin (ADL) and high ash content, the top leaves and top stems of Energycane are not ideal feedstocks for thermochemical conversion. Interestingly, due to a relatively low lignin (ADL) and high hemicellulose content, the top leaves and top stems could be readily converted to biofuels and biobased products using biochemical pathways, especially AD. The bottom leaves are relatively dry and are high in fiber and lignin (ADL) content, which make the bottom leaves ideal for themochemical conversion. However, relatively high ash content in the fiber could be an issue in thermochemical conversion. Similarly, relatively high fiber and lignin (ADL) content make bottom leaves unattractive for biochemical conversion. Thus, leaving bottom leaves in the field is one option, which could result in about 10% of total biomass unavailable for bioenergy/biobased products production. However, leaving the bottom leaves in the field will help recycling carbon and nutrient (bottom leaves are rich in ash), preserving soil moisture (mulching effect) and controlling weeds.

In Napier grass, due to significantly higher fiber (NDF), cellulose and lignin (ADL) content in stems than leaves, stems are a better feedstock for thermochemical conversion than leaves. However, ash content could adversely affect the thermochemical conversion. Napier grass leaves, on the other hand, are more amenable to biochemical conversion, especially AD, as leaves are relatively high in hemicellulose and low in lignin (ADL) and cellulose content compared to stems.

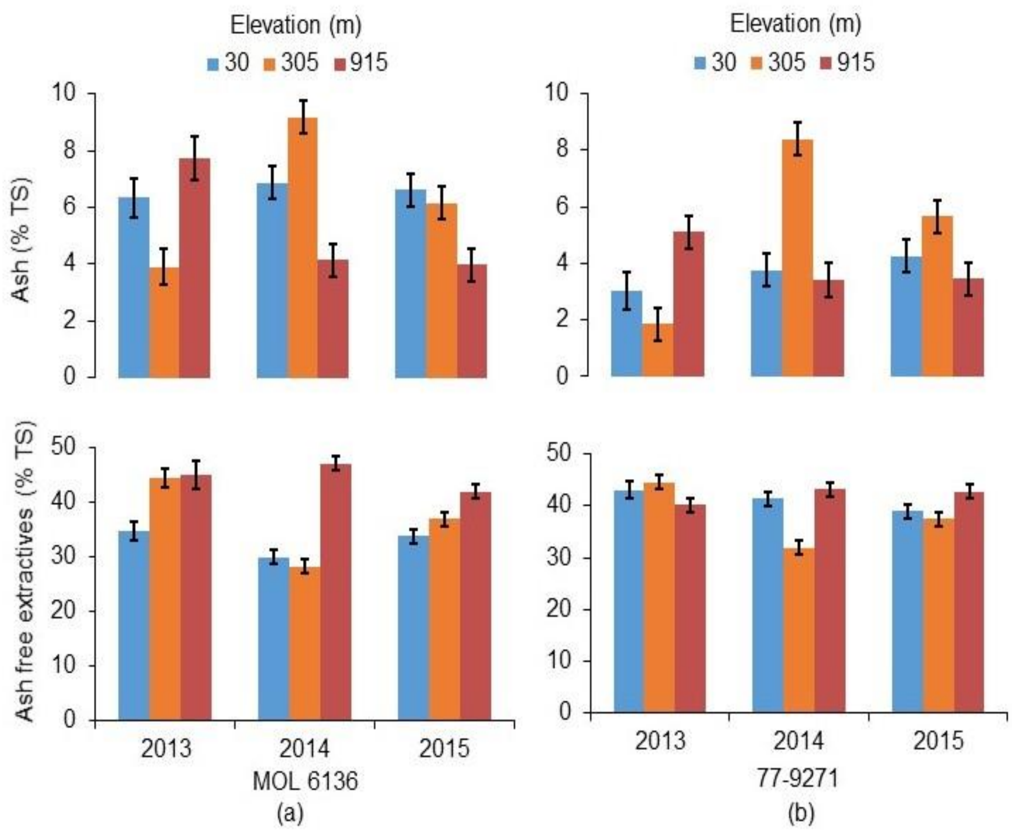

Figure 3.6. Ash and ash free extractives content in Energycane cultivars harvested across the three elevations (30 m, 305 m, and 915 m) and years (2013 through 2015). (a) MOL 6136 cultivar and (b) 77-9271 cultivar

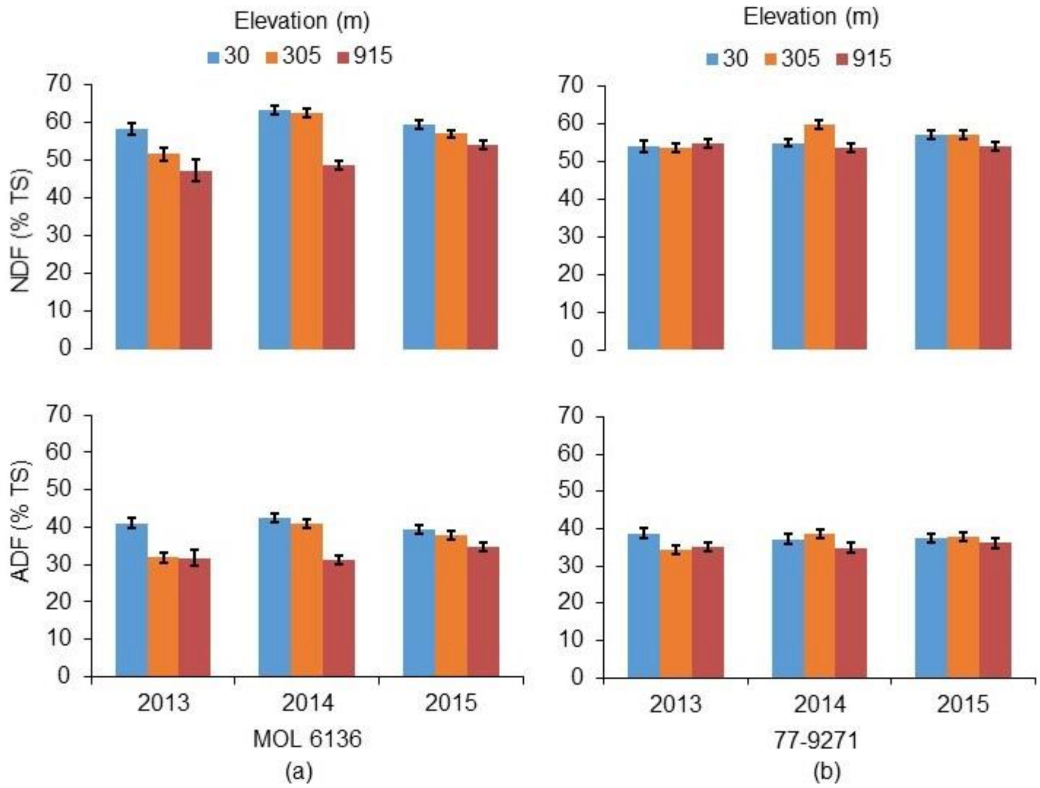

Figure 3.7. NDF and ADF content in Energycane cultivars harvested across the three elevations (30 m, 305 m, and 915 m) and years (2013 through 2015). (a) MOL 6136 cultivar and (b) 77-9271 cultivar

3.2.2 Effects of elevation (location) on biomass composition

In Energycane, ash ($p = 0.0337$), NDF ($p = 0.0020$), ADF ($p = 0.0076$), hemicellulose ($p = 0.0455$), cellulose ($p = 0.0128$) and lignin (ADL) ($p = 0.0037$) content varied significantly across the elevation. NDF, ADF, cellulose and lignin (ADL) content decreased with increasing elevation, while ash and hemicellulose content did not follow a distinct pattern with elevation (Figures 3.6, 3.7 and 3.8). Thus, factors other than elevation influenced ash and hemicellulose content in Energycane. Energycane from the lowest elevation (30 m) had the highest NDF (57.77%), ADF (39.42%), cellulose

(32.63%) and lignin (ADL) (6.78%) content, while Energycane from the highest elevation (915 m) had the lowest NDF (51.97%), ADF (34.01%), cellulose (29.01%) and lignin (ADL) (5.00%) content.

Similarly, in Napier grass, significant differences in ADF ($p = 0.0007$), hemicellulose ($p = 0.0024$) and cellulose ($p = 0.0002$) content were found in the biomass from different elevations. However, differences in ash ($p = 0.1834$), NDF ($p = 0.1917$) and lignin (ADL) ($p = 0.1874$) content were not significant across elevation (Figures 3.9, 3.10 and 3.11). Unlike Energycane, Napier grass from the highest elevation had the highest ADF (50.93%) and cellulose (42.61%) content, while the biomass from the middle elevation (305 m) had the lowest ADF (47.33%) and cellulose (39.39%) content, implying that factors other than elevation governed ADF and cellulose content in Napier grass. Similarly to Energycane, differences in hemicellulose content in Napier grass did not follow any pattern with elevation. The hemicellulose content was highest (23.44%) in the biomass from the middle elevation and was lowest (21.04%) in the biomass from the highest elevation. Thus, factors other than elevation governed the hemicellulose content in Napier grass.

In Energycane, the dry matter yield varied significantly with elevation ($P <.0001$). The highest dry matter yield was found at the highest elevation, followed by the lowest and middle elevations (Figure 3.5). Further, as shown in Table 3.8, Energycane dry matter yield exhibited significant negative correlations with NDF ($r = -0.3390$, $p = 0.0173$) and hemicellulose ($r = -0.4090$, $p = 0.0350$) content. Dry matter yield, however, was not significantly correlated to ash ($r = -0.2490$, $p = 0.0845$), ADF ($r = -0.2030$, $p = 0.1617$), lignin (ADL) ($r = -0.2140$, $p = 0.1403$) and cellulose ($r = -0.1870$, $p = 0.1992$)

content. Additionally, Energycane top and bottom stems biomass yields were negatively correlated with ash, NDF, ADF, hemicellulose and cellulose content in the stems (Table 3.7). In Energycane, the proportion of stem biomass to total biomass (76.72%) was substantially higher than the proportion of leaf biomass (23.28%) (Figure 3.16). Additionally, ash, NDF, ADF, hemicellulose and cellulose content was significantly higher in leaves than stems of Energycane (Table 3.5). The negative correlation of dry matter yield with ash, NDF, ADF, cellulose and hemicellulose content could be due to (i) a higher proportion of stem biomass than leaf biomass, (ii) lower ash, NDF, ADF, hemicellulose and cellulose content in stems than leaves, and (iii) negative correlations of stems dry matter yields with ash, NDF, ADF, hemicellulose and cellulose content in the stems. Thus, the variation in the biomass composition across elevation could be related to difference in dry matter yield.

Similarly to Energycane, Napier grass dry matter yield varied significantly ($p <.0001$) with elevation. The biomass yield was highest at the highest elevation and lowest at the middle elevation (Figure 3.5). Unlike Energycane, Napier grass dry matter yield had a significant positive correlation with ADF ($r = 0.7900$, $p <.0001$), NDF ($r = 0.4860$, $p = 0.0031$), lignin (ADL) ($r = 0.3600$, $p = 0.0337$) and cellulose ($r = 0.8530$, $p <.0001$) content, and a negative correlation with ash ($r = -0.4050$, $p = 0.0159$) and hemicellulose ($r = -0.5820$, $p = 0.0002$) content (Table 3.8). Compared to other elevations, ADF, NDF, cellulose and lignin (ADL) content in biomass, in general, were relatively higher at the elevation with comparatively higher dry matter yield, while hemicellulose and ash content in the biomass were highest at the elevation with the lowest dry matter yield, and vice versa.

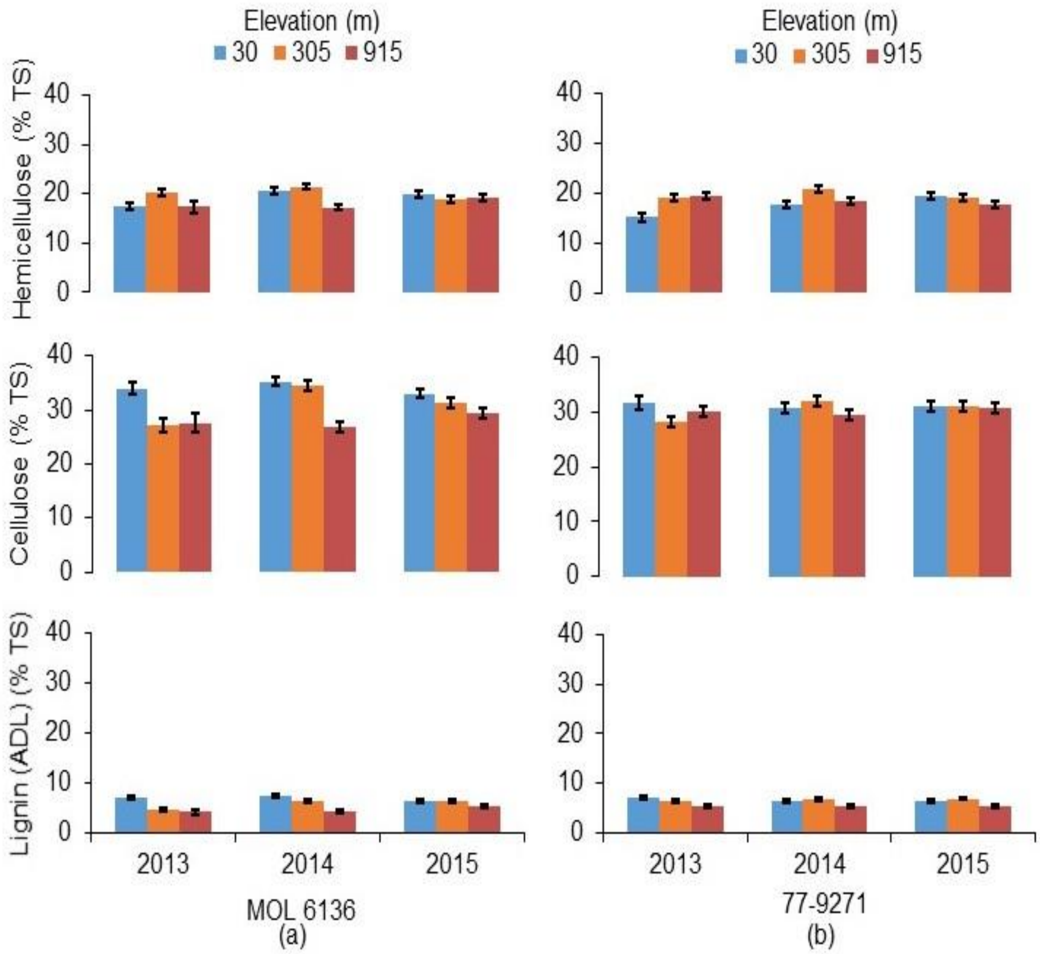

Figure 3.8. Hemicellulose, cellulose and lignin (ADL) content in the Energycane cultivars harvested across the three elevations (30 m, 305 m, and 915 m) and years (2013 through 2015). (a) MOL 6136 cultivar and (b) 77-9271 cultivar

Table 3.8. Pearson correlation between dry matter yield and composition of Energycane and Napier grass

Variable/by variable	Dry matter yield, Energycane (n = 49)		Dry matter yield, Napier grass (n = 35)	
	r	P value	r	P value
Ash	-0.2490	0.0845	-0.4047	0.0159
NDF	-0.3388	0.0173	0.4860	0.0031
ADF	-0.2030	0.1617	0.7895	<.0001
ADL	-0.2137	0.1403	0.3599	0.0337
Hemicellulose	-0.4090	0.0035	-0.5816	0.0002
Cellulose	-0.1866	0.1992	0.8532	<.0001
Ash free extractives	0.3343	0.0189	-0.2099	0.2261

*r = correlation coefficient

In Napier grass, like Energycane, the proportion of stem biomass (64.53%) was substantially higher than the proportion of leaf biomass (35.46%) (Figure 3.16). Additionally, NDF, ADF, cellulose and lignin (ADL) content in stems of Napier grass was significantly higher than in leaves (Table 3.6). The stem dry matter yield had a negative correlation with ash and hemicellulose content, and a positive correlation with NDF, ADF and cellulose content in stems (Table 3.7). The positive correlation of dry matter yield with NDF, ADF, cellulose and lignin (ADL) content, and negative correlation with hemicellulose and ash content could be due to (i) a higher proportion of stem biomass than leaf biomass, (ii) higher NDF, ADF, cellulose and lignin (ADL) content in stems than in leaves, and (iii) a positive correlation of stem dry matter yield with NDF, ADF and cellulose content, and a negative correlation with ash and hemicellulose content in stems. Thus, the variation in biomass composition across elevations could be due to differences in the dry matter yield across elevations.

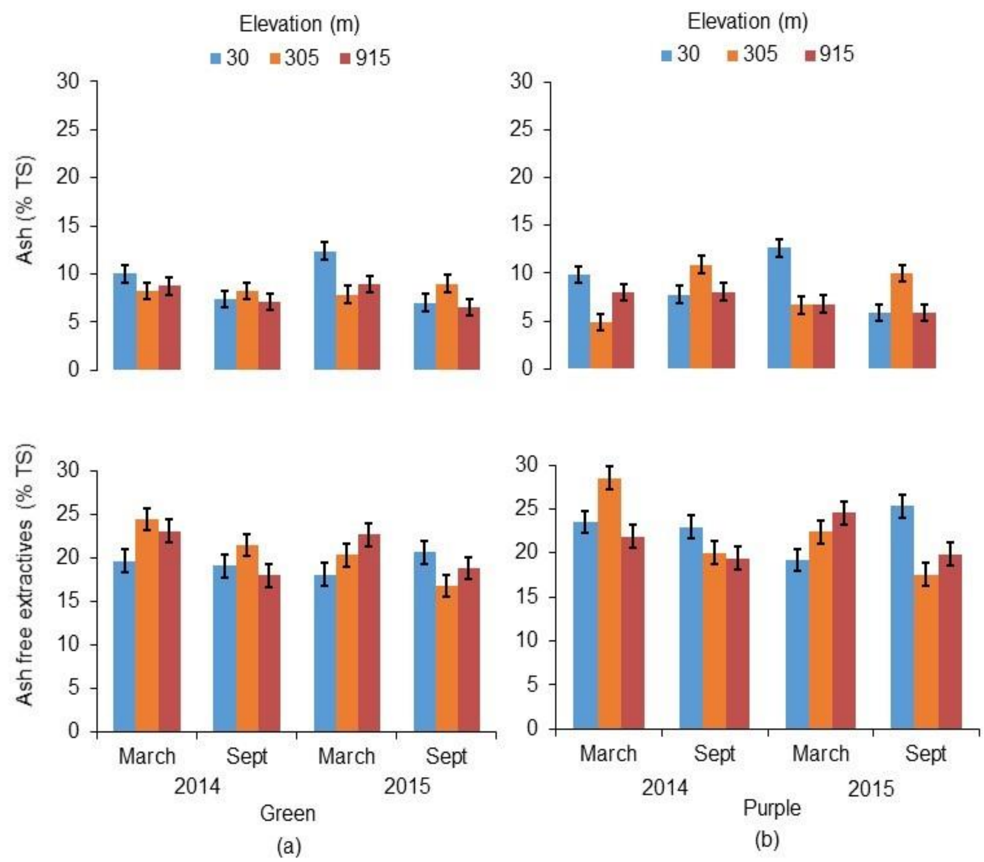

Figure 3.9. Ash and ash free extractive content in Napier grass cultivars harvested across the three elevations (30 m, 305 m, and 915 m), two years (2014 and 2015) and two seasons (March and September). (a) Green cultivar and (b) Purple cultivar

Xue et al. (2011) reported a significant positive correlation of dry matter yield of perennial grasses, such as switchgrass, tall and intermediate wheatgrass and big bluestem with NDF, ADF and cellulose content, and a negative correlation with ash content. The authors also reported no significant correlation of dry matter yield with hemicellulose content. Thus, the biomass composition across elevations differed with the dry matter yield. In Napier grass, the elevation that had the higher dry matter yield provided the more fibrous biomass with lower ash content. Thus, the elevation with higher dry matter yield not only provided more feedstocks, but also provided better biomass quality for thermochemical conversion. In Energycane, the elevation with the highest dry matter yield provided the biomass with the lowest fiber and ash content.

3.2.3 Effects of cultivar on biomass composition

In Napier grass, no significant difference in ash ($p = 0.9112$), ADF ($p = 0.1132$), hemicellulose ($p = 0.0777$) and lignin (ADL) ($p = 0.8122$) content was found between green and purple cultivars. However, the green cultivar had significantly higher NDF ($p = 0.0014$) and cellulose ($p = 0.0157$) content than the purple cultivar. Except for ash content ($p = 0.0027$), there was no significant difference in NDF ($p = 0.5636$), ADF ($p = 0.7691$), hemicellulose ($p = 0.0720$), cellulose ($p = 0.3619$) and lignin (ADL) ($p = 0.0818$) content between the two cultivars of Energycane. The MOL 6136 cultivar had 41.20% higher ash content than the 77-9271 cultivar. Because of similar fiber composition between the cultivars and comparatively lower ash content in the 77-9271 cultivar than the MOL 6136 cultivar, the 77-9271 cultivar of Energycane could be a better feedstock than MOL 6136 cultivar for both thermochemical and biochemical conversion processes.

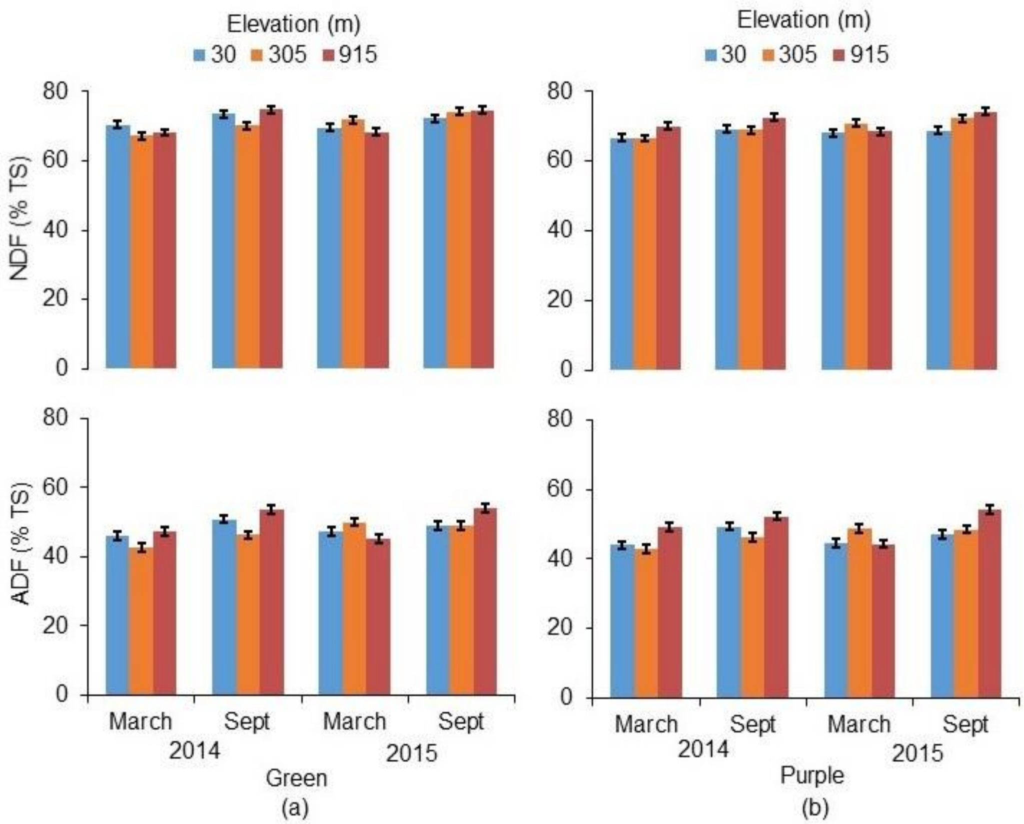

Figure 3.10. NDF and ADF content in Napier grass cultivars harvested across the three elevations (30 m, 305 m, and 915 m), two years (2014 and 2015) and two seasons (March and September). (a) Green cultivar and (b) Purple cultivar

In Napier grass, since the green cultivar had significantly higher fiber (NDF) content than the purple cultivar and there was no significance difference in ash content, the green cultivar could be a better feedstock for thermochemical conversion than the purple cultivar. Also, since the green cultivar was significantly higher in cellulose content, but not significantly different in lignin (ADL) content from the purple cultivar, the green cultivar could yield more fermentable sugar per unit biomass than the purple cultivar during biochemical conversion.

In both Energycane and Napier grass, cultivar by harvest year interactions were not significant for the parameters examined. In Napier grass, cultivar by elevation interaction was significant only for NDF content ($p = 0.0239$). At the lowest elevation, the green cultivar had a significantly higher NDF content (72.21 ± 0.61%) compared to the purple cultivar (68.71 ± 0.61%), while NDF content was not significantly different between cultivars at other elevations studied. In Energycane, cultivar by elevation interaction was significant for ADF ($p = 0.0132$), lignin (ADL) ($p = 0.0243$), hemicellulose ($p = 0.0329$), cellulose ($p = 0.0131$) and ash free extractives ($p = 0.0008$) content. The cultivar by elevation interaction could be mainly attributed to the significant cultivar by elevation interaction for proportion of stem dry matter yield to total dry matter yield where the 77-9271 cultivar had the highest proportion of stem dry matter yield at 30 m elevation (80.35%) while the MOL 6136 cultivar had the lowest proportion of stem dry matter yield to total dry matter yield at 30 m elevation (73.34%). Hemicellulose content was significantly higher in MOL 6136 cultivar than 77-9271 cultivar at 30 m elevation, while ash free extractive content was significantly higher in 77-9271 cultivar than MOL 6136 cultivar at 30 m elevation.

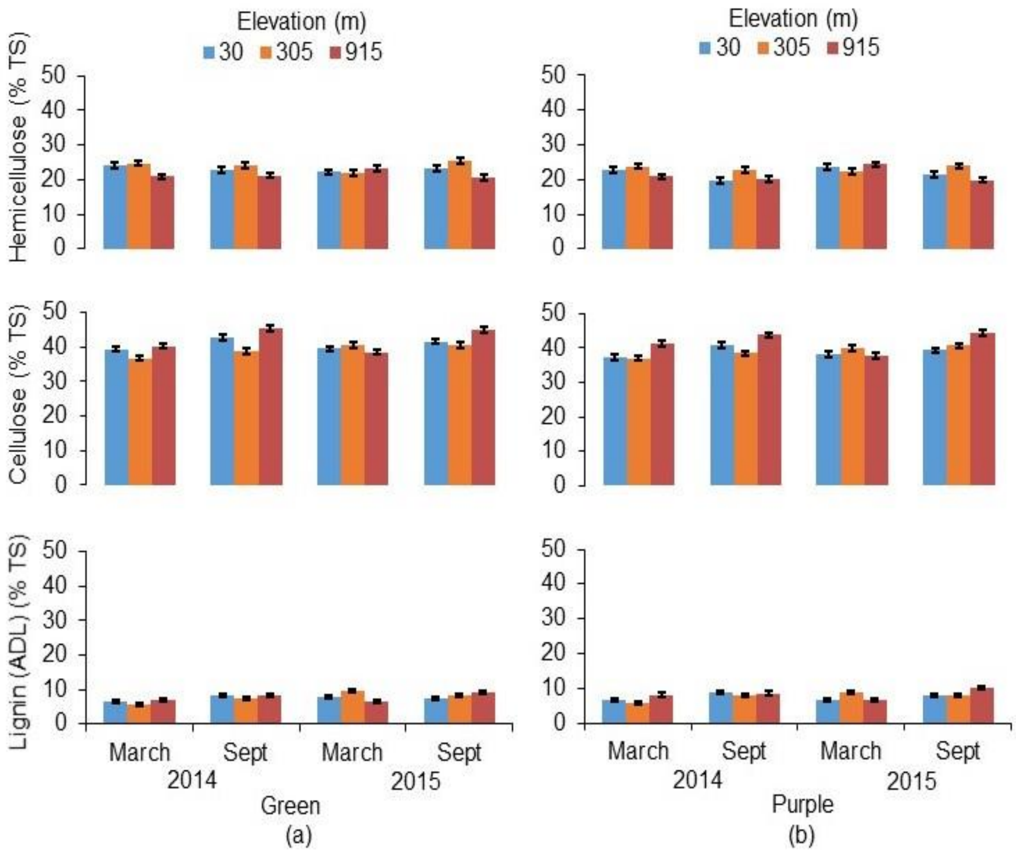

Figure 3.11. Hemicellulose, cellulose and lignin (ADL) content in Napier grass cultivars harvested across the three elevations (30 m, 305 m, and 915 m), two years (2014 and 2015) and two seasons (March and September). (a) Green cultivar and (b) Purple cultivar

3.2.4 Effects of harvest season on biomass composition

There was a significant difference in the composition of Napier grass harvested in March and September. More specifically, compared to the March harvest, the September harvest had significantly higher NDF ($p <.0001$), ADF ($p <.0001$), cellulose ($p <.0001$) and lignin (ADL) ($p = 0.0003$) content, while the ash content was significantly ($p = 0.0111$) lower in the September harvest than in the March harvest. The hemicellulose content, however, did not differ significantly ($p = 0.0690$) between the March and September harvests. Moreover, the dry matter yield was significantly higher ($p <.0001$) in September harvest compared to March harvest. The difference in the Napier grass dry matter yield between the March and September harvests could be attributed to the difference in climatic conditions in two harvest seasons. During the crop growing season for the September harvest, the average monthly air temperature (22.40 ºC) and solar radiation (20.61 MJ m^{-2} day^{-1}) were higher compared to the air temperature (20.32 ºC) and solar radiation (15.46 MJ m^{-2} day^{-1}) during the growing duration for the March harvest. Ferraris et al. (1986) reported an increase in dry matter yield of various accessions of Napier grass studied in Canberra, Australia with increasing temperature, and maximum dry matter yield was achieved at 29.7 or 32.7 ºC. Additionally, the dry matter yield was higher during the seasons with higher solar radiation such as Spring (27.40 MJ m^{-2} day^{-1}) and Summer (23.40 MJ m^{-2} day^{-1}) compared to Autumn (10.50 MJ m^{-2} day^{-1}). Thus, as discussed earlier, the difference in biomass composition between the harvest seasons could be attributed to the difference in the dry matter yields.

Since the March harvest was significantly lower in fiber [NDF, ADF, cellulose and lignin (ADL)] content, and higher in ash content than the September harvest, the

March harvest is more suitable for biochemical conversion than the September harvest. On the other hand, the September harvest, which was significantly higher in fiber [NDF, ADF, cellulose and lignin (ADL)] content and lower in ash content than the March harvest, is more amenable to thermochemical conversion than the March harvest. If both harvests are used for biological conversion processes, different pretreatment conditions will be required for different harvests to maximize biomass to biofuel and biobased conversion efficiency.

3.2.5 Effects of harvest year on biomass composition

In Energycane, except for lignin (ADL) content ($p = 0.1369$), harvest years had significant effects on ash ($p = 0.0039$), NDF ($p = 0.0023$), ADF ($p = 0.0233$), hemicellulose ($p = 0.0312$) and cellulose ($p = 0.0210$) content. The lowest ash (4.66%), NDF (53.25%), ADF (35.52%), hemicellulose (18.20%) and cellulose (29.75%) content was found in the 2013 harvest, while the values for these parameters were highest for the 2014 harvest. Compared to the 2013 harvest, ash, NDF, ADF, hemicellulose and cellulose content increased by 21.68%, 7.14%, 5.97%, 6.65% and 5.95%, respectively, in 2014.

The variation in biomass composition of Energycane with harvest year did not follow the correlation with dry matter yield as explained earlier. Since Energycane dry matter yield had a negative correlation with ash, NDF, ADF, lignin (ADL), hemicellulose and cellulose content, higher dry matter yields in 2014 and 2015 harvests than 2013 harvest should result in lower ash, NDF, ADF, ADL, hemicellulose and cellulose content during the 2014 and 2015 harvests than the 2013 harvest. However, ash, NDF, ADF, lignin (ADL), hemicellulose and cellulose content in 2013 harvest were lower than in 2014 and 2015 harvests despite lower dry matter yield in 2013.

The variation in the composition of the Energycane harvested across the three years could be due to differences in the proportion of the stem and leaf biomass in the total biomass in each year. The proportion of stem biomass was highest for the 2013 harvest [80.85%; bottom stem (70.84%) and top stem (10.01%)] followed by the 2015 [76.35%; bottom stem (70.15%) and top stem (6.20%)] and 2014 [72.94%; bottom stem (62.81%) and top stem (10.13%)] harvests. Since ash, NDF, ADF, cellulose and hemicellulose content was significantly less in Energycane stems than leaves, the increase in proportion of stem biomass would decrease ash, NDF, ADF, cellulose and hemicellulose content in the biomass. The proportion of stem biomass had a significant negative correlation with ash ($r = -0.6355$, $p <.0001$), NDF ($r = -0.5867$, $p <.0001$), ADF ($r = -0.4343$, $p < 0.0018$), hemicellulose ($r = -0.5401$, $p = 0.0001$) and cellulose ($r = -0.5200$, $p = 0.0001$) content in the biomass. However, there was no significant correlation between the proportion of stem biomass and ash content in the biomass ($r = -0.1245$, $p = 0.3940$). The high values of ash, NDF, ADF, hemicellulose and cellulose content in the biomass harvested in 2014 compared to 2013 and 2015 could be due to the low proportion of stem biomass (72.94%) in 2014. Similarly, the highest proportion of stem biomass (80.15%) and the lowest content of ash, NDF, ADF, hemicellulose and cellulose were found in the 2013 harvest. For the 2015 harvest, the proportion of stem biomass (76.35%), and ash, NDF, ADF, hemicellulose and cellulose content in biomass, were between the 2014 and 2013 levels. Thus, in addition to dry matter yield, the difference in the proportion of stem or leaf biomass significantly affected the overall biomass composition. Therefore, separating the energy crop into plant parts (leaves and stems) would provide more consistency in biomass composition with dry matter yield.

In Napier grass, NDF ($p = 0.0449$) and lignin (ADL) ($p = 0.0037$) content differed significantly between the harvest years. However, no significant difference was observed in ash ($p = 0.4277$), ADF ($p = 0.1538$), hemicellulose ($p = 0.3852$) and cellulose ($p = 0.8934$) content between harvest years. Although, statistically significantly different, NDF and lignin (ADL) content in Napier grass increased only by 1.30% and 6.39%, respectively, from the harvests of 2014 to 2015. Dry matter yield of Napier grass did not differ significantly ($p = 0.3204$) across the harvest years. Takara and Khanal (2015) and Drielak (2015) also reported consistency in biomass composition of mature green Napier grass (at 6 to 8 months of age) harvested across the years in Hawaii, USA.

3.2.6 Effects of crop type on biomass composition

The ash, NDF, ADF, hemicellulose, cellulose and lignin (ADL) content in Napier grass harvested across the elevations, years and seasons varied from 7.36 - 8.76%, 68.90 - 72.19%, 46.04 - 50.93%, 21.04 - 23.44%, 38.85 - 42.61% and 7.20 - 8.32%, respectively. Similarly, Energycane had ash, NDF, ADF, hemicellulose, cellulose and lignin (ADL) content in the range of 4.32 - 6.10%, 51.97 - 57.77%, 34.01 - 39.42%, 18.20 - 19.98%, 29.01 - 32.63% and 5.00 - 6.78%, respectively. The composition of Napier grass found in this study is in the range previously reported by Van Man and Wiktorsson (2003) and Rengsirikul et al. (2011). Similarly, cellulose, hemicellulose, lignin (ADL) and ash content in the Energycane is in the range previously reported by Knoll et al. (2013).

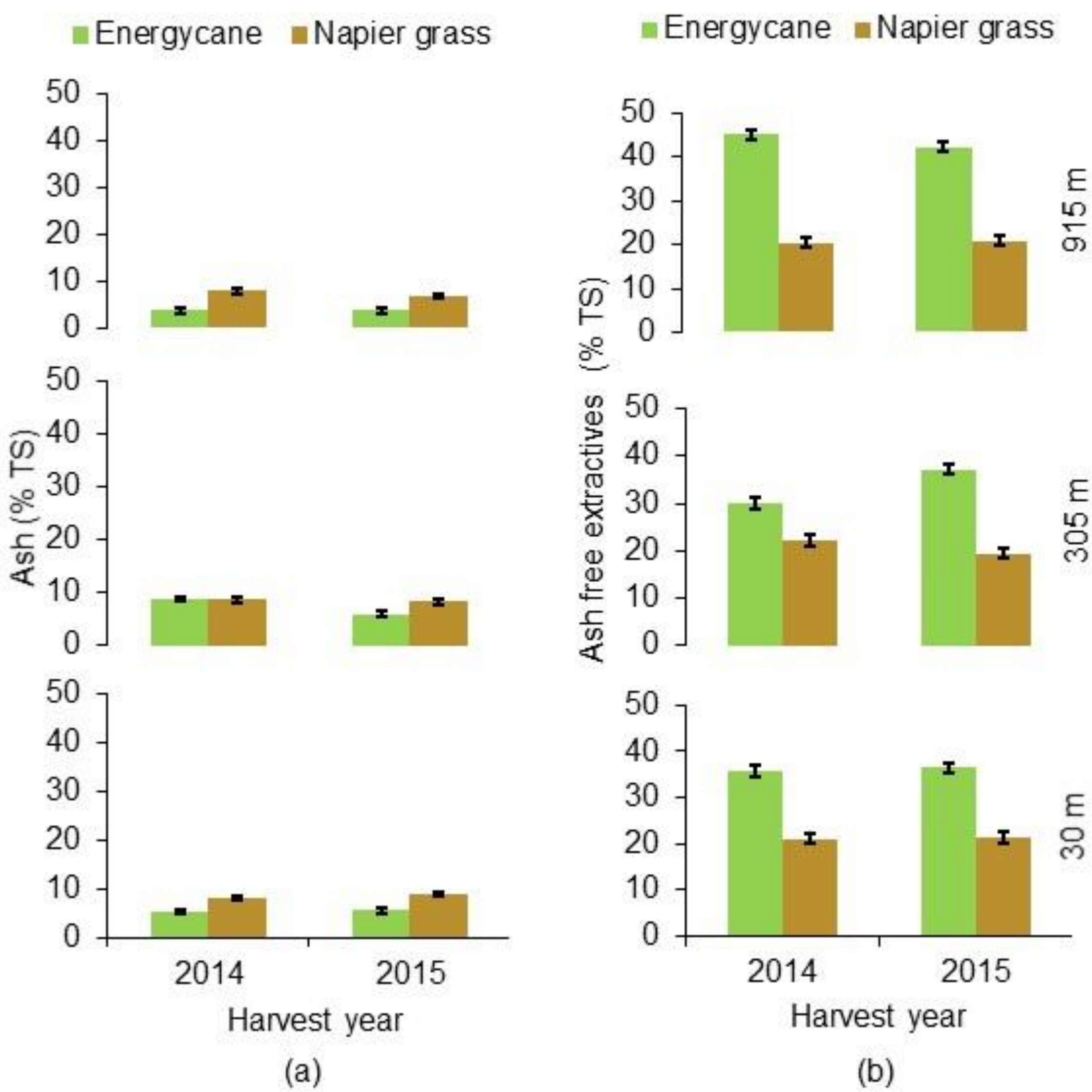

Figure 3.12. Composition of Energycane and Napier grass across the three elevations (30 m, 305 m and 915 m) and two harvest years (2014 and 2015). (a) ash content (% TS) and (b) ash free extractives content (% TS)

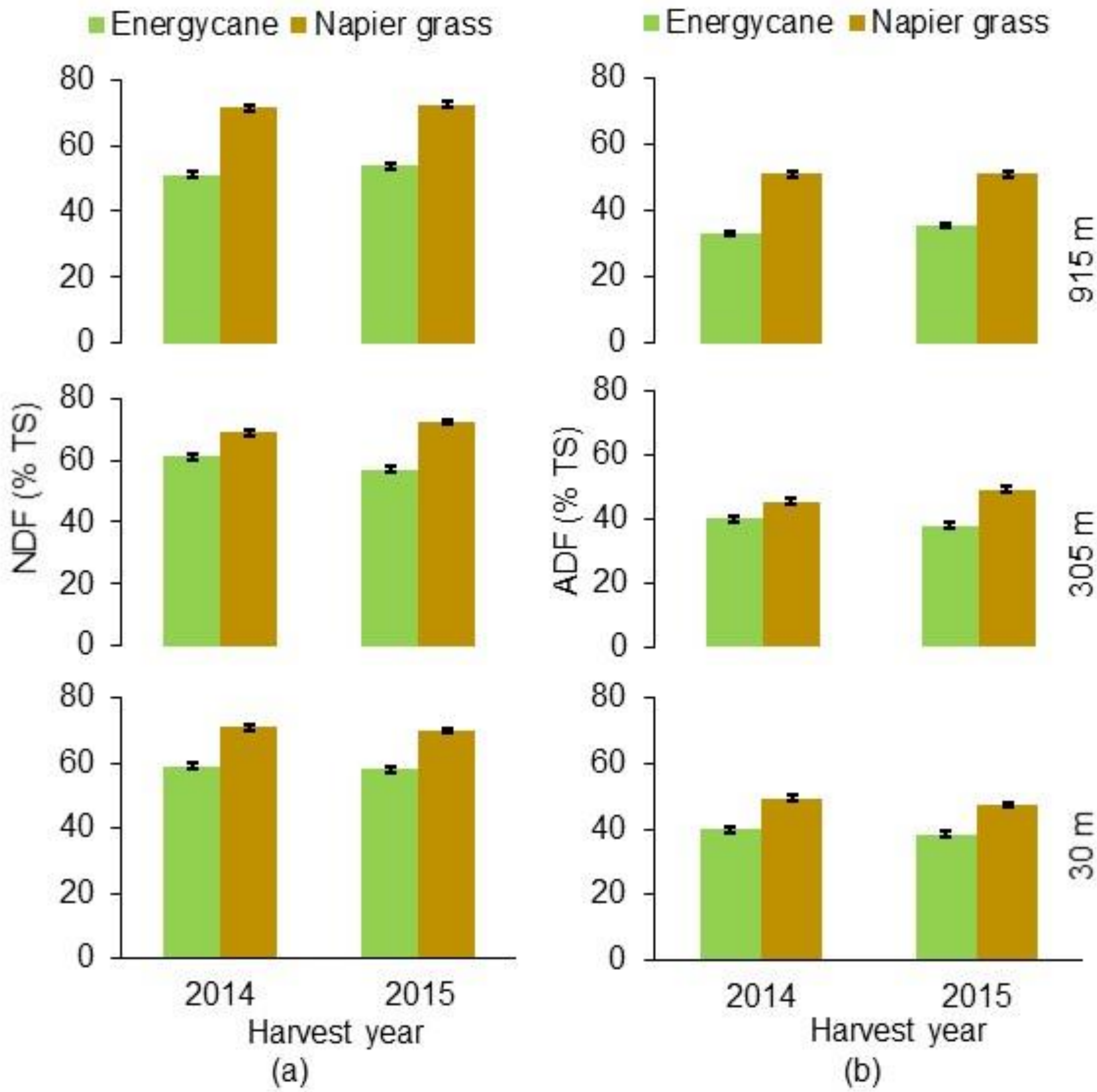

Figure 3.13. Composition of Energycane and Napier grass across the three elevations (30 m, 305 m and 915 m) and two harvest years (2014 and 2015). (a) NDF content (% TS) and (b) ADF content (% TS)

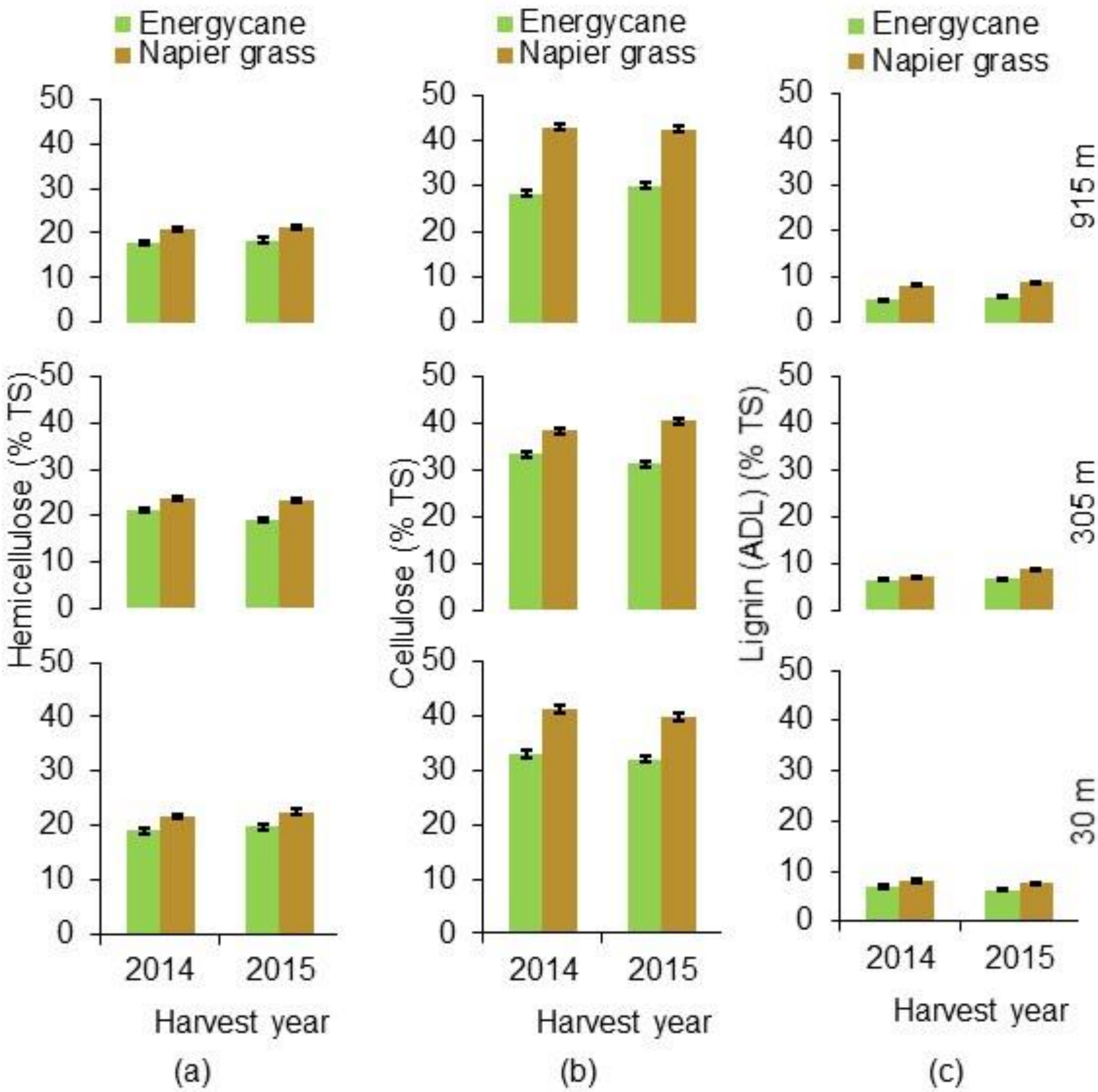

Figure 3.14. Composition of Energycane and Napier grass across the elevations (30 m, 305 m and 915 m) and the harvest years (2014 and 2015) (a) hemicellulose content (% TS), (b) cellulose content (% TS), and (c) lignin (ADL) content (% TS)

There was a significant difference between Napier grass and Energycane in ash ($p < .0001$), NDF ($p < .0001$), ADF ($p < .0001$), hemicellulose ($p < .0001$), cellulose ($p < .0001$) and lignin (ADL) ($p < .0001$) content. In overall, Napier grass had 47.60%, 25.28%, 30.45%, 15.23%, 30.44%, and 30.64 %, higher ash, NDF, ADF, hemicellulose, cellulose and lignin (ADL) content, respectively, than Energycane. Additionally, the crop type by elevation interaction was significant for ash ($p = 0.0257$), ash free extractive ($p = 0.0001$), NDF ($p = 0.0008$), ADF ($p = 0.0011$), cellulose ($p = 0.0010$) and lignin (ADL) content (Figures 3.12, 3.13 and 3.14). In Napier grass, ash, NDF, lignin (ADL) and ash free extractives content was not significantly different across the elevation, but, compared to other elevation, Energycane had significantly lower ash, NDF and lignin (ADL) content, and significantly higher ash free extractive content at the highest elevation. Similarly, compared to other elevation, ADF and cellulose content in Napier grass was significantly higher at the highest elevation, while ADF and cellulose content in Energycane was significantly lower at the highest elevation. The dry matter yields of Napier grass and Energycane were highest at the highest elevation and were lowest at the middle elevation. Dry matter yields of Energycane and Napier grass had contrasting correlations with ash, ash free extractives, NDF, ADF, cellulose, hemicellulose and lignin (ADL) content in the biomass. For example, Energycane dry matter yield was negatively correlated with ash, NDF, ADF, cellulose, hemicellulose and lignin (ADL) content, while Napier grass dry matter yield was positively correlated with ash, NDF, ADF, cellulose, hemicellulose and lignin (ADL) content in the biomass (Table 3.8). Thus, the differences in dry matter yields across the elevations and contrasting correlations of dry matter yield with ash, NDF, ADF, cellulose, hemicellulose and lignin (ADL) content between the two crops could have resulted in the significant crop type by elevation interactions.

Except for ash content for 2014 harvest at 305 m elevation, for all elevations and harvest years, ash, NDF, ADF, hemicellulose, cellulose and lignin (ADL) content were higher in Napier grass than Energycane (Figures 3.12, 3.13 and 3.14). The higher ash, NDF, ADF, hemicellulose, cellulose and lignin (ADL) content in Napier grass than Energycane was also reported by Na et al. (2014b). In terms of whole crop composition, the higher fiber (i.e., NDF) and lignin (ADL) content in Napier grass than Energycane may favor thermochemical conversion of Napier grass rather than Energycane. Due to the higher ash content in Napier grass than in Energycane, thermochemical conversion of Napier grass, however, could be more problematic than that of Energycane. Compared to Napier grass, Energycane could be more amenable to biochemical conversion due to higher soluble total solids (including soluble sugars) and lower lignin (ADL) content. Due to a higher concentration of lignin (ADL) in the fiber (i.e., lignin (ADL) content expressed as % of NDF), fiber portion of the Energycane, however, could be recalcitrant to biochemical conversion.

3.3 Results and Discussion: Objective 3

3.3.1 Specific methane yield

3.3.1.1 Specific methane yield from different plant parts

Specific methane yields from plant parts of both energy crops differed significantly ($p = <.0001$). In Energycane, the top stems had the highest specific methane yield followed by the top leaves, bottom stems and bottom leaves. When compared between the stems and leaves, the stems had significantly higher ($p = 0.0316$) specific methane yields than leaves. In Napier grass, the specific methane yield of leaves was significantly higher ($p <.0001$) than stems. Compared to stems, leaves had 44.64% higher specific methane yields.

The difference in the specific methane yields among the plant parts was attributed to the difference in composition of the plant parts. The specific methane yields from Energycane plant parts showed significant negative correlation with lignin (ADL) ($r = -0.78$, $p <.0001$), ADF ($r = -0.54$, $p <.0001$) and cellulose ($r = -0.39$, $p = 0.0007$) content in the biomass. The low specific methane yield from the bottom leaves and the bottom stems compared to the top leaves and the top stems could be due to the higher lignin (ADL) content in the bottom leaves and the bottom stems compared to the top stems and the top leaves. Besides, the bottom leaves had higher ADF and cellulose content compared to the other plant parts. The lower specific methane yield from the bottom stems compared to the top stems and the top leaves despite of lower ADF and cellulose content in the bottom stems than the top stems and the top leaves could be due to significant negative correlation ($r = -0.69$, $p <.0001$) between ash free extractives content in the biomass and methane content in the biogas. Energycane bottom stems had the highest ash free extractives than rest of the plant parts. The methane content in the biogas from Energycane bottom stems was the lowest compared to the methane content in the biogas produced from other plant parts. The rapid degradation of the ash free extractives (including nonstructural carbohydrates) present in the bottom stems could have resulted rapid volatile fatty acids build-up in the digester, and inhibited the methanogenesis, and ultimately reduced the methane content in the biogas. Kandel et al. (2013) also observed only 25 - 30% methane content in the biogas produced from the young reed canary grass compared to above 50% methane in the biogas produced from the mature reed canary grass during the first week of AD. The difference in methane content in the biogas was also attributed to inhibition of methanogenesis due to rapid degradation of readily fermentable components of the young biomass. Additionally, when expressed as a relative concentration in fiber, Energycane bottom stems had the highest cellulose and lignin (ADL) content compared to other parts of the plant.

Similarly, in Napier grass, the specific methane yield showed strong negative correlation with lignin (ADL) ($r = -0.93$, $p < .0001$), ADF ($r = -0.91$, $p < .0001$), cellulose ($r = -0.86$, $p < .0001$) and NDF ($r = -0.83$, $p < .0001$) content, while the specific methane yield had significant positive correlation with hemicellulose ($r = 0.77$, $p < .0001$) and ash free extractives ($r = 0.73$, $p < .0001$) content in the biomass. Thus, the higher specific methane yield from leaves of Napier grass than stems was attributed to the comparatively higher hemicellulose and ash free extractives, and the lower lignin (ADL), cellulose, ADF and NDF content in leaves than stems of Napier grass.

Table 3.9. Effects of plant parts and cultivars on specific and total methane yields from Energycane harvested across the three elevations

Factors	Methane content (%)	Specific methane yield [Nm^3 (kg VS_{added})$^{-1}$]	Total methane yield (Nm^3 ha^{-1} $year^{-1}$)
Plant part			
Leaf, Bottom	56.55 ± 0.18^a	0.199 ± 0.004^a	829 ± 242^a
Leaf, Top	56.06 ± 0.18^a	0.251 ± 0.004^b	$1,004 \pm 242^a$
Stem, Bottom	52.61 ± 0.18^b	0.230 ± 0.004^c	$6,318 \pm 242^b$
Stem, Top	53.53 ± 0.18^c	0.257 ± 0.004^b	598 ± 242^a
Plant part: stems and leaves combined			
Leaf	56.32 ± 0.15^a	0.224 ± 0.003^a	$1,833 \pm 383^a$
Stem	52.68 ± 0.15^b	0.232 ± 0.003^b	$6,916 \pm 383^b$
Cultivar			
77-9271	53.51 ± 0.25^a	0.228 ± 0.002^a	$8,476 \pm 791^a$
MOL 6136	53.30 ± 0.25^a	0.231 ± 0.002^a	$9,022 \pm 791^a$
Elevation (m)			
30	53.26 ± 0.26^a	0.227 ± 0.003^{ab}	$5,545 \pm 1,103^a$
305	53.78 ± 0.26^a	0.224 ± 0.003^b	$8,698 \pm 1,103^{ab}$
915	53.18 ± 0.26^a	0.239 ± 0.003^a	$12,004 \pm 1,103^b$
P value			
Plant part	<.0001	<.0001	<.0001
Plant part: stems and leaves combined	<.0001	0.0316	<.0001
Cultivar	0.6062	0.2765	0.5824
Elevation (m)	0.2990	0.0256	0.0174

*Least square mean values ± standard error (n = 18 for plant part, n = 9 for cultivar, n = 6 for elevation) followed by a different letter within a column and within a factor are significantly different at the $P \leq 0.05$ level

Several studies have reported higher cellulose and lignin content, and lower hemicellulose content in stems than in leaves of several energy crops, such as mature reed canary grass (Kandel et al., 2013), Miscanthus spp. (Wahid et al., 2015) and Napier grass (Ansah et al., 2010). Monlau et al. (2012) also found higher specific methane yield of leaves than stems of Giant reed grass and Jerusalem artichoke. Similarly, higher specific methane yields of leaves than stems of two Miscanthus spp. have been reported during 31 days AD (Wahid et al., 2015). The authors also reported significant difference in the specific methane yield from biomass harvested at different dates, where the later harvest produced significantly lower specific methane yield compared to the early harvest. The significant decrease in the specific methane yield with increase in harvest date was attributed to the increase in lignin content in the later harvest than the early harvest. Similarly, several studies reported the significant negative correlation between lignin content in biomass and the specific methane yield from different energy crops and agri-residues (Li et al., 2013; Rath et al., 2013; Dandikas et al., 2014). In addition to being recalcitrant to biological degradation, lignin also restricts microbes (or enzymes and chemicals) accessing the cellulose and hemicellulose. Thus, higher concentration of lignin in the biomass not only reduces the amount of degradable constituent (per unit biomass) during AD, but also limits the degradation of other components such as cellulose and hemicellulose, and ultimately reduces the overall specific methane yield. Hemicellulose is more favorable to biological degradation than cellulose, which resulted the positive correlation of hemicellulose content in biomass with specific methane yield. Higher biodegradability of hemicellulose as compared to cellulose could be due to (i) lower degree of polymerization of hemicellulose compared to cellulose, (ii) branched structure of hemicellulose in contrast to linear structure of cellulose, and (iii) amorphous nature of hemicellulose, while cellulose is highly crystalline structure.

Table 3.10. Effects of plant parts, cultivars and harvest seasons on the specific and total methane yields from Napier grass harvested across the three elevations

Factors	Methane content (%)	Specific methane yield [Nm^3 (kg VS_{added})$^{-1}$]	Total methane yield (Nm^3 ha^{-1} $year^{-1}$)
Plant part			
Leaf	55.44 ± 0.14[a]	0.243 ± 0.002[a]	1,180 ± 79[a]
Stem	56.21 ± 0.14[b]	0.168 ± 0.002[b]	1,608 ± 79[b]
Harvest season			
Mar	55.64 ± 0.19[a]	0.201 ± 0.003[a]	2,317 ± 184[a]
Sept	55.95 ± 0.19[a]	0.188 ± 0.003[b]	3,259 ± 184[b]
Cultivar			
Green	56.14 ± 0.18[a]	0.192 ± 0.003[a]	5,898 ± 415[a]
Purple	55.63 ± 0.18[a]	0.192 ± 0.003[a]	5,253 ± 415[a]
Elevation (m)			
30	56.01 ± 0.25[a]	0.197 ± 0.004[a]	3,566 ± 526[a]
305	56.35 ± 0.25[a]	0.191 ± 0.004[a]	3,856 ± 526[a]
915	55.29 ± 0.25[a]	0.187 ± 0.004[a]	9,304 ± 526[b]
P value			
Plant Part	0.0014	<.0001	<.0001
Harvest season	0.3542	0.0178	0.0007
Cultivar	0.0533	0.9824	0.2981
Elevation (m)	0.0556	0.3483	0.0004

*Least square mean values ± standard error (n = 36 for plant part, n = 18 for cultivar, n = 12 for elevation) followed by a different letter within a column and within a factor are significantly different at the $p \leq 0.05$ level

3.3.1.2 Effects of cultivar, harvest season, elevation and crop type on specific methane yield

There was no significant difference in the specific methane yield between the cultivars of Energycane ($p = 0.2765$) and Napier grass ($p = 0.9824$). Cultivar by elevation interaction for specific methane yield was significant for Napier grass ($p = 0.0261$), but was not significant for Energycane ($p = 0.4121$). Although ANOVA showed a significant cultivar by elevation interaction for specific methane yield from Napier grass, the least square means separation using Tukey HSD test did not show any significant difference among specific methane yields from the two cultivars across the elevation at $\alpha = 0.05$. In Napier grass, purple cultivar had the highest specific methane yield value at the elevation of 305 m and the lowest at the elevation of 915 m, while the green cultivar showed the highest specific methane yield at the elevation of 915 m and the lowest at the elevation of 305 m. The difference in specific methane yields between the cultivars could be due to the difference in lignin (ADL) content with the elevation. The lignin (ADL) content in purple cultivar was highest at the highest elevation (915 m), while in green cultivar the lignin (ADL) content was highest at the middle elevation (305 m).

In Napier grass, biomass harvested in March and September differed significantly ($p = 0.0178$) in specific methane yield. The March harvest had 6.9% higher specific methane yield compared to the September harvest. The higher specific methane yield of the March harvest compared to the September harvest was attributed to the lower NDF, ADF, cellulose and lignin (ADL), and higher ash free extractives content in the March harvest than in the September harvest.

Significant difference ($p = 0.0256$) in specific methane yield from Energycane was found across the elevation. The highest elevation had the highest specific methane yield followed by the lowest and middle elevations. The highest specific methane yield of Energycane at the highest elevation could be due to the lower NDF, ADF, cellulose and lignin (ADL) content in the biomass from the highest elevation compared to the biomass from the middle and the lowest elevations. Although effect of elevation on specific methane yield from Napier grass was not significant ($p = 0.3483$), decreasing trend of specific methane yield was found with increasing elevation. The decrease in specific methane yield from Napier grass with increasing elevation could be attributed to the increasing concentration of NDF, ADF, and cellulose content in Napier grass collected from the higher elevations.

When compared between Energycane and Napier grass, crop type showed significant ($p <.0001$) effect on specific methane yield. Average specific methane yield from Energycane [0.230 ± 0.002 Nm^3 methane (kg VS_{added})$^{-1}$] was higher than from Napier grass [0.192 ± 0.002 Nm^3 methane (kg VS_{added})$^{-1}$]. In overall, the Energycane had 19.79% higher specific methane yield than Napier grass. For all elevations examined, specific methane yield from Energycane was higher than that from Napier grass and there was no significant ($p = 0.0531$) crop type by elevation interaction for the specific methane yield.

The specific methane yields from Napier grass and Energycane were reported to vary from 0.190 - 0.340 Nm^3 methane (kg VS_{added})$^{-1}$ and 0.228 - 0.298 Nm^3 methane (kg VS_{added})$^{-1}$, respectively (Chynoweth et al., 1993). The specific methane yields from Napier grass and Energycane obtained in this study were in the lower range of the reported value. This could be due to the shorter incubation time used in this study compared to the incubation time reported (Chynoweth et al., 1993). The specific methane yields from Napier grass and Energycane were, however, within the range of values reported for several perennial grasses, such as Timothy clover grass, reed canary grass (Lehtomäki et al., 2008), sorghum, Giant reed grass (Monlau et al., 2012; Barbanti et al., 2014), Miscanthus (Wahid et al., 2015; Herrmann et al., 2016; Kiesel and Lewandowski, 2017), Switchgrass (Li et al., 2013; Barbanti et al., 2014), tall wheatgrass, Jerusalem artichoke, Cup plant, and country mallow (Herrmann et al., 2016).

The specific methane yields from Napier grass and Energycane were less than the specific methane yield from maize silage (major feedstocks used for biogas production in Europe), which has been reported to vary widely from 0.196 - 0.557 Nm^3 methane (kg VS_{added})$^{-1}$ (Gao et al., 2012; Rath et al., 2013; Mayer et al., 2014; Herrmann et al., 2016) with mean value in the range of 0.355 - 0.419 Nm^3 methane (kg VS_{added})$^{-1}$ (Rath et al., 2013; Mayer et al., 2014; Herrmann et al., 2016). The higher specific methane yield from maize silage was attributed to its favorable biomass composition for biological degradation such as, high starch (26 – 36 % TS) and low fiber (i.e., NDF = 35 – 45 % TS, ADF = 18 – 27 % TS) and lignin (ADL) (1 – 3 % TS) content (Rath et al., 2013; Herrmann et al., 2016). However, due to the higher dry matter yields of Napier grass and Energycane compared to maize, Napier grass and Energycane may result higher overall total methane yield per hectare per year. Besides, as perennial plants, Energycane and Napier grass are likely to require less inputs compared to maize. Thus, Energycane and Napier grass could potentially result in higher net energy yield than maize as a feedstock for biogas production.

3.3.1.3 Effect of incubation time on specific methane yield

Plant parts not only had significant effect on the specific methane yield but also on the rate of methane production. The effect of incubation time on specific methane yield from different plant parts of Energycane and Napier grass is presented in Figure 3.15. Compared to the specific methane yield obtained at 90 days of incubation time, 82% of methane yield from the bottom stems of Energycane was achieved within the first 15 days, while only 60%, 63%, and 79% methane yields were produced from the bottom leaves, top leaves and top stems, respectively, within the first 15 days. In Napier grass, stems produced nearly 65% of specific methane yield within the first 15 days, while the value for leaves was 63%.

During 35 days of incubation time, which is the standard incubation time of Hohenheim Biogas Yield Test (HBT) method, the bottom stems of Energycane produced 94% of specific methane yield compared to 92% from top stems and 86 - 88% of specific methane yield obtained from other parts of the plant. The same incubation time resulted in 85 - 86% of specific methane yields from stems and leaves of Napier grass.

Early peak in the specific methane yield from the bottom stems of Energycane was due to the higher amount of ash free extractives (readily degradable nonstructural soluble constituents) present in the bottom stems of Energycane. As shown in Figure 3.15, within 35 days of incubation, cumulative specific methane yield from the bottom stems reached plateau (specific methane yield increased only by 6% when incubation time was increased from 35 to 90 days). The small increase in the specific methane yield from the bottom stems while increasing the incubation time could be due to very slow degradation of the fiber component of the bottom stems. The relatively higher concentration of lignin (ADL) in the fiber component of the bottom stems could have attributed to the recalcitrance of the bottom stems fiber to biological degradation. On the other hand, slow but consistent increase in the cumulative specific methane yields from the top and the bottom leaves were observed, which could be due to slow but steady degradation of the fiber components of the respective plant parts.

Kandel et al. (2013) reported an early peak in the cumulative specific methane yield from young sorghum than mature sorghum. A small but continuous increase in the cumulative specific methane yield was observed from stems of mature biomass with increasing incubation time when the specific methane yield from leaves had reached the maximum suggesting slower biodegradation of fiber components of the mature stems. Wahid et al. (2015) also found that the major part of methane came from both leaf (78 - 85%) and stem (69 - 78%) fractions of two Miscanthus genotypes within the first 31 days of AD. The high conversion rate at the initial incubation period was due to rapid degradation of readily biodegradable components present in the biomass. The lower lignin content in leaves compared to stems was believed to contribute to the faster degradation of leaves than stems at the beginning of the batch test. However, continuous methane production was found from stems towards the end of the batch test when leaves fraction reached the maximum methane production (Wahid et al., 2015).

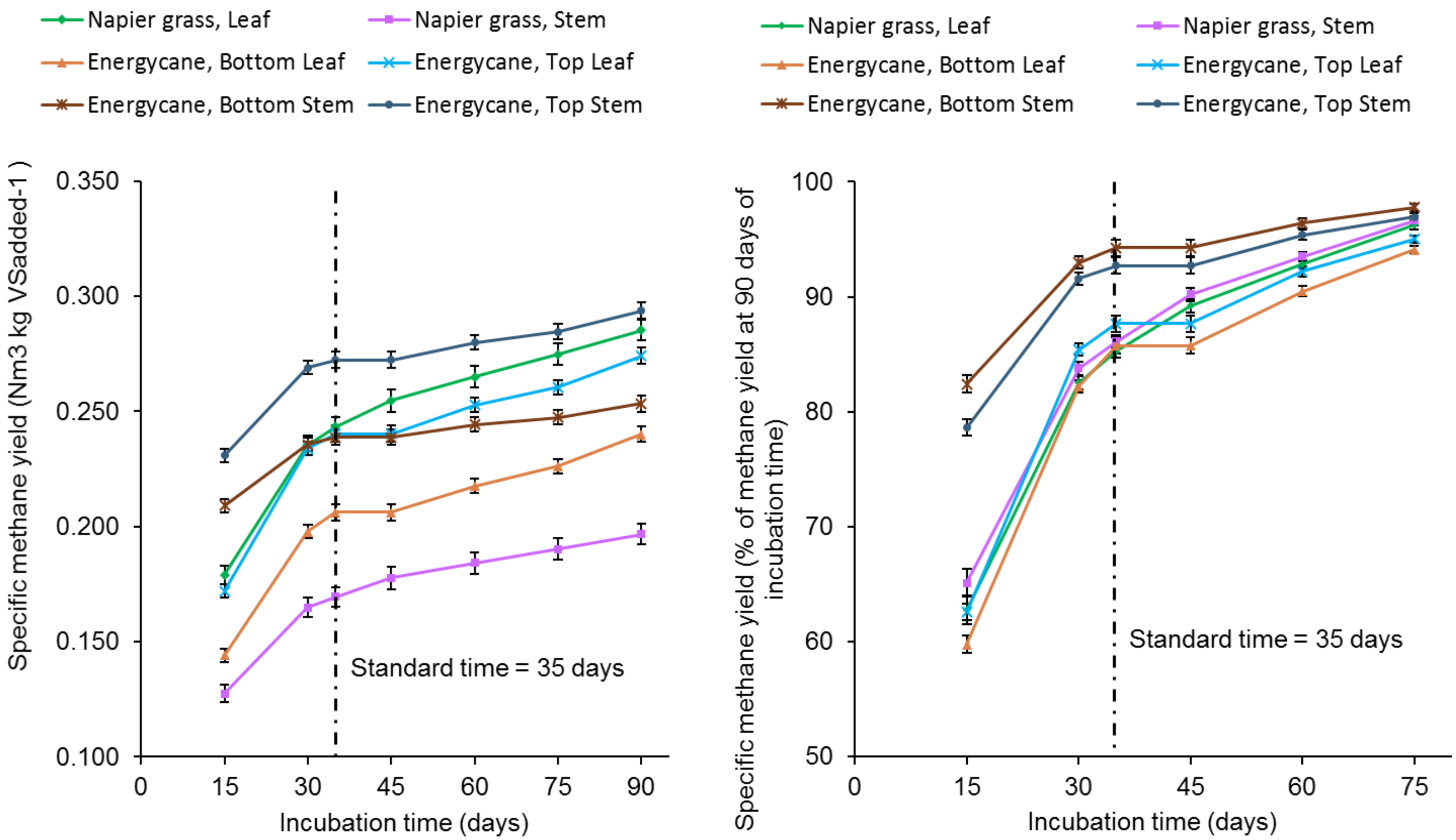

Figure 3.15. Effect of incubation time on cumulative specific methane yields from plant parts of Energycane and Napier grass

3.3.2 Total methane yield

3.3.2.1 Effects of cultivar, harvest season, elevation and crop type on total methane yield per hectare per year

The difference in total methane yield per hectare between cultivars was not significant for both Energycane ($p = 0.5824$) and Napier grass ($p = 0.2981$). Although the difference was not significant, total methane yield per hectare from MOL 6136 cultivar was slightly higher (numerically) compared to 77-9271 cultivar. The difference in total dry matter yields between the cultivar was mainly attributed to the difference in the total methane yield per hectare. In Napier grass, despite of similar specific methane yield from both cultivars, total methane yield from green cultivar was slightly higher (numerically) than from purple cultivar. Similarly to Energycane cultivars, the higher total methane yield from green cultivar of Napier grass was primarily governed by its higher dry matter yield. Furthermore, the cultivar by elevation interaction for total methane yield per hectare was not significant for both Energycane ($p = 0.4171$) and Napier grass ($p = 0.1387$).

Although the specific methane yield from the March harvest was significantly higher than that from the September harvest, the total methane yield per hectare from the September harvest was significantly higher ($p = 0.0007$) than from the March harvest. The total methane yield from the September harvest was 40.65% higher than that from the March harvest. The September harvest, which accounted for 60.02% of total annual dry matter yield, contributed 58.45% of total methane yield. On the other hand, the March harvest accounted for 39.98% and 41.55% of total annual dry matter and total methane yields, respectively.

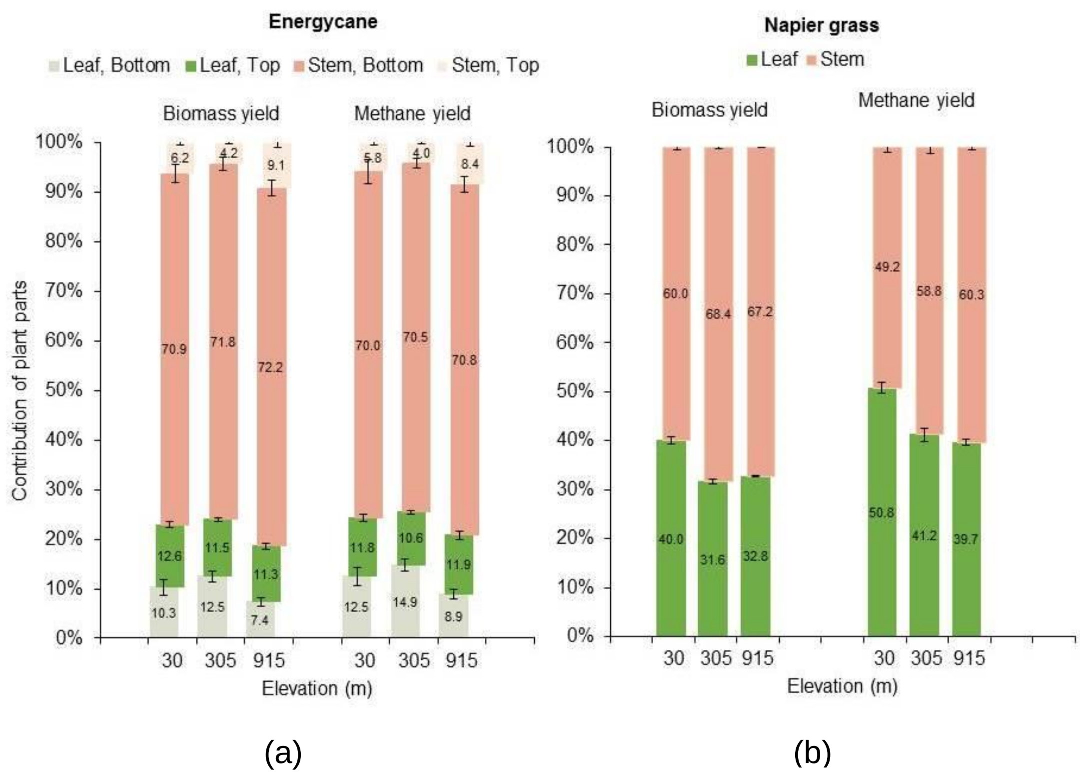

Figure 3.16. Contribution of plant parts to total dry matter and methane yields. (a) Energycane and (b) Napier grass

The season by elevation interaction for the total methane yield per hectare was significant ($p = 0.0050$) which was due to the slightly higher dry matter yield in the March harvest than in the September harvest at the 305 m elevation, unlike significantly higher dry matter yield during the September harvest than the March harvest at other elevations. However, the season by cultivar ($p = 0.3749$) and season by cultivar and elevation ($p = 0.4630$) interactions were not significant.

There was significant difference in total methane yields from both Energycane ($p = 0.0174$) and Napier grass ($p = 0.0004$) across the elevations. The total methane yield from Energycane varied from 5,545 - 12,004 Nm^3 methane ha^{-1} $year^{-1}$ across the elevations, while the total methane yield from Napier grass varied from 3,566 - 9,304 Nm^3 methane ha^{-1} $year^{-1}$. For both energy crops, the highest total methane yield was obtained at the highest elevation, while the total methane yield per hectare was the lowest at the lowest elevation. For Energycane, the significantly higher total methane yield at the highest elevation was both due to the combined effects of the highest dry matter yield at the highest elevation and the highest specific methane yield from the biomass harvested at the highest elevation. In Napier grass, the total methane yield for different elevation was primarily governed by the difference in the dry matter yield across the elevation and not much by the difference in the specific methane yield.

Energycane and Napier grass differed significantly ($p = 0.0016$) in total methane yield per hectare. On an average across the elevations, total methane yield per hectare from Energycane (8,749 ± 494 Nm^3 methane ha^{-1} $year^{-1}$) was 56.93% higher than from Napier grass (5,575 ± 494 Nm^3 methane ha^{-1} $year^{-1}$). However, no significant ($p = 0.1253$) crop type by elevation interaction was observed for the total methane yield per hectare.

The average total methane yield from Napier grass was higher than or similar to the reported total methane yields from various dedicated energy crops, such as Miscanthus spp. (4,468 - 5,825 Nm^3 methane ha^{-1} $year^{-1}$) (Mayer et al., 2014; Wahid et al., 2015), reed canary grass (3,735 - 5,430 Nm^3 methane ha^{-1} $year^{-1}$) (Kandel et al., 2013), sorghum (2,500 - 5,800 Nm^3 methane ha^{-1} $year^{-1}$) (Mahmood and Honermeier, 2012; Seppala et al., 2013; Mayer et al., 2014) and rye (3,972 - 4,812 Nm^3 methane ha^{-1} $year^{-1}$) (Hübner et al., 2011). The average total methane yield from Energycane, however, was higher than the values reported for most of the energy crops.

Similarly to specific methane yield from maize silage, depending on the locations, cultivars and crop management practices, the total methane yield per hectare from maize has been reported to widely vary from 2,130 - 10,200 Nm^3 methane ha^{-1} $year^{-1}$ (Amon et al., 2007a; Amon et al., 2007b; Schittenhelm, 2008; Bruni et al., 2010; Oslaj et al., 2010; Seppälä et al., 2012; Mayer et al., 2014). However, in countries such as Denmark and Germany where maize silage is the most commonly used feedstock in the commercial anaerobic digesters, average total methane yield per hectare ranged from 5,500 - 9,400 Nm^3 methane ha^{-1} $year^{-1}$ (Schittenhelm, 2008; Bruni et al., 2010). Thus, an average total methane yield from Napier grass falls at the lower end of the range of the total methane yield from maize. However, the total methane yield achieved from Napier grass at the 915 m elevation falls at the higher end of the total methane yield per hectare from maize. The average total methane yield from Energycane was at the higher end of the range value reported for maize. Additionally, the total methane yield obtained from Energycane at the highest elevation was substantially higher than the reported total methane yield potential from maize.

More importantly, as perennial plants, Energycane and Napier grass are likely to require less inputs for crop management compared to maize. Thus, Energycane and Napier grass potentially result in higher net energy yield than maize as a feedstock for biogas production. Besides, Energycane and Napier grass could be cultivated in the marginal land with minimum reduction in dry matter yield, while maize requires fertile land for producing a good harvest. More importantly, perennial crops are reported to offer better ecological and environmental benefits compared to annual crops (Xue et al., 2011; Sumiyoshi et al., 2016).

Furthermore, as of now, the specific methane yield from Napier grass and Energycane are low compared to maize silage, which is most likely due to the poor digestibility of the fiber components of the biomass. Ensiling freshly harvested biomass, as practiced in most of the energy crop-based commercial digesters, could improve the fiber digestibility and ultimately enhance the specific methane yield from Napier grass and Energycane. Additionally, since significant amount of fiber rich solid residue will be produced following AD, it will provide substrate for downstream processing into diverse products of interest following wide array of conversion technologies, such as gasification, torrefaction and hydrothermal liquefaction (Surendra et al., 2015; Chayanone et al., 2017).

3.3.2.2 Contribution of plant part in total methane yield per hectare

In Energycane, although the specific methane yield was the highest from the top stems and the top leaves, the total methane yield per hectare was highest from the bottom stems, followed by the top leaves, bottom leaves and top stems. The contribution of plant parts to total dry matter and methane yields are summarized in Figure 3.16. The bottom stems contributed 70.42% of dry matter and 71.65% of total methane yields, while the top stems, top leaves, and bottom leaves, contributed 6.07%, 11.42% and 12.09% of dry matter yield, respectively, with respective total methane yield of 6.51%, 11.79% and 10.05%. When compared between leaves and stems of Energycane, stems had significantly higher ($p < .0001$) methane yield per hectare than leaves. Energycane stems accounted for 76.49% of dry matter and 78.16% of total methane yields compared to leaves, which accounted for 23.51% of dry matter and 21.84% of total methane yields.

Although the specific methane yield from Napier grass leaves was significantly higher than from Napier grass stems, the total methane yield from stems was significantly higher ($p < .0001$) than from leaves. The stems biomass, which accounted for the 65.21% of dry matter yield, contributed 56.09% of total methane yield. Leaves, on the other hand, contributed 34.79% of dry matter and 43.91% of total methane yields.

Strong influence of dry matter yield on total methane yield per hectare than specific methane yield was reported by other researchers (Kandel et al., 2013; Wahid et al., 2015). The higher contribution of stems fraction to total methane yield than leaves has been reported for reed canary grass (Kandel et al., 2013) and Miscanthus (Wahid et al., 2015). Wahid et al. (2015) reported the contribution of Miscanthus stems to total methane yield in the range of 54 - 70% compared to 30 - 46% from the leaf fraction.

CHAPTER 4: ENGINEERING IMPLICATIONS

The biomass composition differed significantly with the plant parts and crop types. In Energycane, the top leaves and top stems were relatively high in hemicellulose content and low in lignin (ADL) content, which favors the biochemical conversion. The bottom stems, which account for about 70% of total dry matter yield, was rich in ash free extractive (about 46%) including nonstructural carbohydrates. The high amount of ash free extractives including nonstructural carbohydrates favors the biochemical conversion, while the higher relative concentration of lignin (ADL) content in the fiber component of the bottom stems makes it less favorable to biochemical conversion. The physical and chemical pretreatments intended to deconstruct the structure of fiber component are likely to generate degradation products resulting mainly from the degradation of nonstructural carbohydrates (bottom stems) or hemicellulose (top stems and leaves). Such degradation products, such as hydroxymethyl furfural (HMF) and furfural, ultimately inhibit the downstream biochemical conversion (enzymatic saccharification and fermentation) of the pretreated biomass.

During biochemical methane potential test, over 80% of methane yield was obtained from plant parts, especially from the bottom and top stems of Energycane within 30 days of incubation time. However, only minimal increase in methane yield was achieved with increasing the incubation time to 90 days. This was likely due to the rapid hydrolysis and methanogenesis of easily degradable components such as nonstructural carbohydrates in the bottom stems, and hemicellulose in the top leaves and top stems at the beginning of the incubation. The minimum increase in methane yield could be due to the slow degradation of the fiber components including cellulose.

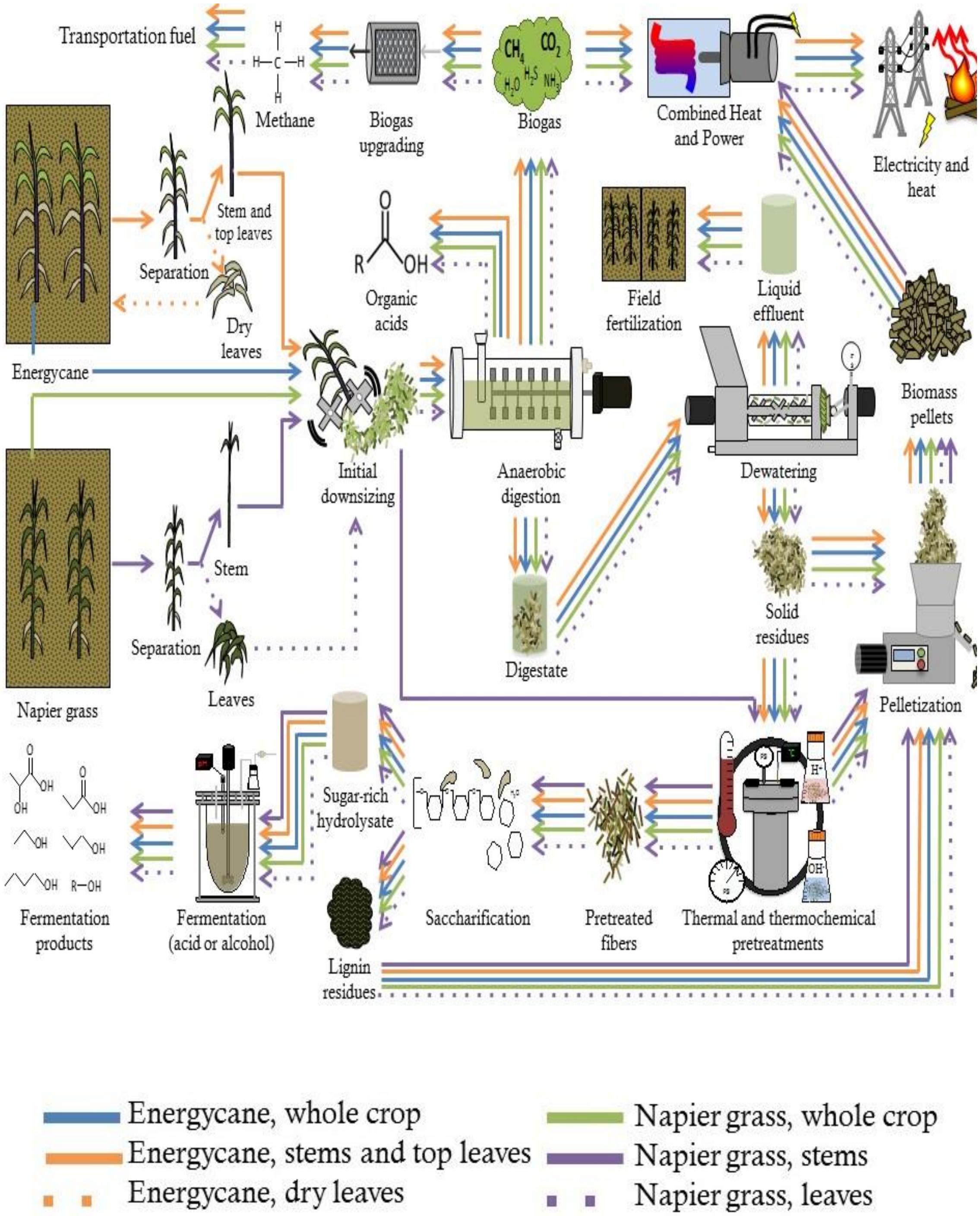

Figure 3.17. Schematics of potential conversion technology for the energy crops for producing biofuels and biobased products.

Thus, neither AD nor the thermal and chemical pretreatment techniques alone is likely to efficiently utilize the biomass components for generating biofuels and biobased products. However, combination of AD with other thermochemical conversion technologies, such as torrefaction, hydrothermal carbonization, pyrolysis, or thermochemical pretreatment followed by cellulosic biofuel production could be an attractive strategy for efficiently converting residual biomass (digestate) components into biofuel and biobased products. During AD, the nonstructural carbohydrates and hemicellulose are converted to organic acids or biogas using naturally occurring mixed anaerobic microbial consortia. The produced organic acids could be used as platform chemical for producing diverse biobased products including advanced biofuels, while the biogas could be converted to heat and electricity using combined heat and power (CHP) unit or upgraded to biomethane and used as a natural gas substitute. The undigested fiber (digestate) can be used either for cellulosic biofuel (or biobased product) generation or themochemical conversion as discussed in Figure 3.17 (Surendra et al., 2015; Sawatdeenarunat et al., 2017). Incorporation of the AD at the front end will generate the energy in the form of heat and electricity, which could meet the energy demand for downstream processing of digestate.

In Energycane, the dried bottom leaves, which had comparatively high ash content and are comparatively more recalcitrant to biochemical conversion [due to high lignin (ADL) and cellulose content] than other plant components, could be left in the field which would help recycling carbon and nutrients to the soil, preserving soil moisture and controlling weed problem. The rest of the plant parts could be processed in two steps, AD followed by either cellulosic biofuel production via biochemical means or thermochemical conversion pathways, such as torrefaction, hydrothermal carbonization and pyrolysis. In Napier grass, AD of leaves appeared to be more attractive than stems due to higher specific methane yield from leaves than stems, while stems were comparatively recalcitrant to AD. Since hemicellulose content in the leaves are higher than in the stems of Napier grass, separating leaves from stems will likely to reduce the production of inhibitory compound such as furfural during thermochemical pretreatment of Napier grass. Additionally, during thermochemical conversion, such as pyrolysis, torrefaction and hydrothermal liquefaction of Napier grass stems, lower hemicellulose and higher cellulose and lignin (ADL) content in the stems compared to leaves, could result in the better mass and energy recoveries compared to processing of whole crop. Thus, two-step process with AD at the front end followed by cellulosic biofuel production or thermochemical conversion could efficiently convert Energycane and Napier grass into biofuel and biobased products.

CHAPTER 5: CONCLUSION

5.1 Conclusion: Objective 1

Napier grass composition showed statistically significant variations with respect to maturation, thereby affecting the digestibility of biomass for methane production. Although the specific methane yield was higher for the biomass harvested at early ages of growth, harvesting and processing biomass frequently may not always result in the highest net energy yield (per unit area per year) for a commercial biorefinery. Thus, to maximize the net energy yield from a given planting area, a complete life cycle assessment (LCA) must be conducted and include parameters such as feedstock age and preprocessing strategies (i.e., sieving regimes).

5.2 Conclusion: Objective 2

This study examined the ash content and fiber composition of different parts of two high yielding tropical energy crops harvested across three locations and harvest years. Significant differences were found in the composition of energy crops among the plant parts within the crop types and between the crop types. In general, Energycane leaves were higher in ash and fiber content than stems, with the highest ash and fiber content found in the bottom leaves making them the least favorable component of Energycane for biological conversion. The bottom stems of Energycane were rich in ash-free extractives (including soluble sugars), which can be readily converted to biofuels and biobased products via biochemical means, especially AD. The fiber component of the bottom stems, however, was high in lignin content, which could be an excellent feedstock for thermochemical conversion. The top leaves and top stems, which were relatively high in hemicellulose and low in lignin (ADL) content, are more amenable to biochemical conversion, especially AD. Unlike Energycane, Napier grass stems were higher in fiber content, including cellulose and lignin (ADL) content, than leaves. The relatively higher hemicellulose and lower lignin (ADL) content makes Napier grass leaves better feedstock for biochemical conversion such as AD than stems. Additionally, whole crop composition within the crop type varied with elevations and harvest years (and seasons in Napier grass) based on the biomass yield and proportion of stem and leaf biomass. Energycane biomass yield and proportion of stem biomass were negatively correlated with the ash and fiber content, while biomass yield and proportion of stem biomass of Napier grass were correlated positively with fiber content and negatively with ash content. Thus, the higher biomass yield of Energycane (and/or higher proportion of stem biomass) offers a feedstock more favorable for biochemical conversion, while the higher biomass yield of Napier grass (and/or higher proportion of stem biomass) not only provides more feedstock, but also provides good biomass quality for thermochemical conversion. For all the years and elevations covered in this study, fiber and ash content were higher in Napier grass than Energycane. Overall, Energycane appears to be better feedstock than Napier grass for biochemical conversion, including AD, whereas Napier grass seems more suitable for thermochemical conversion than Energycane.

5.3 Conclusion: Objective 3

Significant difference in specific and total methane yields were observed between crop types and plant parts within a crop. Although the specific methane yield is higher from leaves than stems of Napier grass, stem fraction contributed the major portion of the total methane yield than leaves due to a significantly higher dry matter yield of stem component than leaves. In Energycane, both specific and total methane yields from stems were higher than from leaves. However, there was no significant difference in specific and total methane yields between the cultivars within a crop type. In comparison to Napier grass, Energycane had higher both specific and total methane yields. However, selecting Energycane over Napier grass as a feedstock for biogas production should be based on its performance at diverse growing condition, such as marginal agricultural land and under low input condition. The crop selection should also consider the parameters, such as resistance to diseases and pests, and tolerance to stress such as drought and limited nutrient conditions.

CHAPTER 6: FUTURE WORKS

The findings reported in this study is based on the composition analysis and anaerobic digestion of the two candidate energy crops. This study has shown that the composition of the energy crops varies with crop maturity, crop type and plant parts within the crop type, which ultimately affects the anaerobic digestibility for biogas production. However, effects of soil fertility (on biomass composition and subsequent conversion efficiency), other potential crop types and cultivars, and conversion technologies were not covered in this study. Moreover, the biogas yield data was solely based on the small-scale batch study. More importantly, the techno-economic analysis (TEA) and life cycle assessment (LCA) were not considered within this study. Thus, further studies on energy crops for bioenergy and biobased products production should examine the following aspects:

- This study mainly focused on the composition of the selected energy crops. Only the weather data was analyzed to see if the weather conditions affects the yield and composition of the energy crops. The soil fertility aspects, however, was not accounted on this study. Soil fertility, which has significant effects on crop yield, may significantly affect the composition of the energy crops as well. Thus, it is necessary to determine the effects of soil fertility on yields and composition of the energy crops.
- Two cultivars of the selected energy crops were examined for their composition and subsequent anaerobic digestion for biogas production. With rapid crop breeding programs, new cultivars and candidate energy crops could be already out there. Thus, it is necessary to evaluate the performance of the other cultivars and

crop types before selecting the candidate energy crops for a particular location for bioenergy production.

- The biogas yield data was based on the small-scale batch study. Thus, it is necessary to conduct scale-up studies on continuous or semi-continuous feeding mode reflecting the commercial practices of energy crop digestion to optimize the digestion process and conduct TEA and LCA of the energy crop digestion for biogas production.
- Since biomass composition affects the conversion efficiency of the biomass into biofuels and biobased products and significant difference in biomass composition was observed between the crop types and plant parts within the crop type, it is necessary to evaluate the performance of other biomass to biofuel and biobased conversion technologies, such as pyrolysis, gasification, hydrothermal carbonization, hydrothermal liquefaction, cellulosic ethanol production among others to maximize the technical and economic efficiency of biomass to biofuel and/or biobased products conversion.
- Finally, conducting an in-depth TEA and LCA are essential in selecting appropriate energy crop type/cultivar and conversion technologies. Such analyses will be crucial in maximizing economic return with minimal environmental impacts.

APPENDIX A: STATISTICAL ANALYSIS OF EXPERIMENTAL DATA

Table A.1. ANOVA for composition of different plant parts of the Energycane cultivars collected across the three elevations and harvest years

Source	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	10.01	0.0129	18.32	0.0028	15.67	0.0041	8.52	0.0175	24.20	0.0013	5.00	0.0526	31.53	0.0006
Cultivar	22.82	0.0030	12.48	0.0123	0.06	0.8079	0.31	0.5967	0.22	0.6543	0.06	0.8134	13.64	0.0106
Cultivar*Elevation	2.24	0.1867	9.05	0.0155	4.11	0.0753	4.76	0.0578	0.04	0.9623	5.02	0.0522	5.33	0.0480
Plant part	177.42	<.0001	1403.33	<.0001	1423.06	<.0001	383.63	<.0001	966.52	<.0001	520.00	<.0001	64.63	<.0001
Plant part*Elevation	28.30	<.0001	12.67	<.0001	18.48	<.0001	13.23	<.0001	8.03	<.0001	10.25	<.0001	7.64	<.0001
Plant part*Cultivar	0.77	0.5198	4.20	0.0124	10.06	<.0001	6.42	0.0015	4.49	0.0090	9.18	0.0001	3.26	0.0328
Plant part*Elevation*Cultivar	0.92	0.4911	4.15	0.0031	5.87	0.0003	3.33	0.0113	2.14	0.0727	2.81	0.0248	2.03	0.0885
Harvest year	15.13	<.0001	17.24	<.0001	7.85	0.0007	7.51	0.0010	3.68	0.0290	5.86	0.0040	6.80	0.0018

Table A.1. (Continued) ANOVA for composition of different plant parts of the Energycane cultivars collected across the three elevations and harvest years

Source	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Harvest year*Elevation	78.95	<.0001	23.24	<.0001	2.98	0.0232	16.98	<.0001	8.42	<.0001	16.39	<.0001	6.93	<.0001
Harvest year*Cultivar	1.57	0.2141	0.36	0.6977	1.64	0.1989	1.94	0.1498	1.13	0.3266	1.35	0.2644	3.69	0.0288
Harvest year*Plant part	14.17	<.0001	1.15	0.3392	3.89	0.0017	3.39	0.0046	0.70	0.6480	1.79	0.1106	8.74	<.0001
Harvest year*Elevation*Cultivar	0.11	0.9770	3.51	0.0102	4.25	0.0033	4.06	0.0045	1.90	0.1177	3.82	0.0065	2.34	0.0605
Harvest year*Elevation*Plant part	4.45	<.0001	7.04	<.0001	9.39	<.0001	9.05	<.0001	3.89	<.0001	11.28	<.0001	2.44	0.0083
Harvest year*Cultivar*Plant part	2.48	0.0288	1.32	0.2582	1.46	0.2000	1.37	0.2334	0.84	0.5442	0.82	0.5563	2.13	0.0568
Harvest year*Elevation*Cultivar*Plant part	0.84	0.6106	2.42	0.0088	2.20	0.0176	1.71	0.0763	1.41	0.1773	2.17	0.0192	1.78	0.0639

Table A.2. ANOVA for composition of leaves and stems of Energycane cultivars collected across the three elevations and harvest years

Source	Ash		Ash free extractive		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	8.53	0.0257	24.76	0.0014	18.15	0.0028	9.16	0.0149	16.68	0.0034	6.49	0.0317	17.48	0.0030
Cultivar	36.05	0.0008	13.21	0.0109	0.01	0.9264	0.15	0.7081	0.55	0.4877	0.08	0.7832	6.15	0.0487
Cultivar*Elevation	2.86	0.1310	14.42	0.0051	9.10	0.0157	5.00	0.0540	0.86	0.4707	4.34	0.0693	5.28	0.0487
Plant part	603.73	<.0001	2655.86	<.0001	2537.86	<.0001	394.21	<.0001	2104.04	<.0001	762.87	<.0001	3.77	0.0768
Plant part*Elevation	36.71	<.0001	12.92	0.0012	31.24	<.0001	20.81	0.0002	9.74	0.0027	19.52	0.0002	21.88	0.0001
Plant part*Cultivar	1.84	0.1990	7.08	0.0218	15.48	0.0023	6.57	0.0261	4.49	0.0546	9.31	0.0111	1.13	0.3101
Plant part*Elevation*Cultivar	0.83	0.4609	8.20	0.0063	11.03	0.0023	5.46	0.0221	2.75	0.1016	7.09	0.0106	1.90	0.1935
Harvest year	12.11	<.0001	17.04	<.0001	9.24	0.0004	9.78	0.0003	0.10	0.9076	7.41	0.0017	11.26	0.0001
Harvest year*Elevation	57.22	<.0001	27.27	<.0001	4.72	0.0029	10.86	<.0001	5.88	0.0007	9.40	<.0001	10.11	<.0001

Table A.2. (Continued) ANOVA for composition of leaves and stems of Energycane cultivars collected across the three elevations and harvest years

Source	Ash		Ash free extractive		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Harvest year*Cultivar	0.72	0.4933	1.18	0.3178	1.91	0.1604	2.07	0.1387	0.05	0.9472	1.82	0.1732	2.84	0.0690
Harvest Year*Plant part	0.93	0.4038	1.54	0.2256	0.81	0.4529	1.19	0.3127	1.67	0.1994	1.38	0.2632	2.32	0.1106
Harvest year*Elevation*Cultivar	0.03	0.9981	2.79	0.0374	3.88	0.0087	1.65	0.1790	2.46	0.0597	1.41	0.2478	1.35	0.2673
Harvest year*Elevation*Plant part	1.19	0.3278	22.10	<.0001	29.50	<.0001	13.70	<.0001	6.71	0.0003	18.69	<.0001	0.88	0.4837
Harvest year*Cultivar*Plant part	1.85	0.1697	2.56	0.0888	4.35	0.0189	2.21	0.1220	1.06	0.3552	1.61	0.2109	3.28	0.0470
Harvest year*Elevation*Cultivar*Plant part	0.24	0.9148	3.62	0.0122	3.75	0.0103	2.13	0.0934	1.00	0.4205	2.34	0.0697	1.30	0.2829

Table A.3. ANOVA for composition of Energycane cultivars collected across the three elevations and harvest years

Source	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	60.48	0.0337	28.13	0.0012	20.59	0.0020	12.27	0.0076	5.38	0.0455	9.97	0.0128	15.87	0.0037
Cultivar	22.51	0.0027	17.01	0.0066	1.09	0.5636	0.10	0.7691	5.52	0.0720	0.98	0.3619	4.33	0.0818
Cultivar*Elevation	2.36	0.1711	32.20	0.0008	37.18	0.2499	11.14	0.0132	8.26	0.0329	10.18	0.0131	7.34	0.0243
Harvest year	7.15	0.0039	14.49	0.0001	10.02	0.0023	4.57	0.0233	4.16	0.0312	4.70	0.0210	2.19	0.1369
Harvest year*Elevation	25.09	<.0001	16.10	<.0001	6.55	0.0043	7.75	0.0006	7.07	0.0011	7.50	0.0007	5.66	0.0031
Harvest year*Cultivar	2.13	0.1414	1.03	0.3735	2.48	0.1195	1.62	0.2219	0.07	0.9287	1.25	0.3078	2.26	0.1289
Harvest year*Elevation*Cultivar	0.20	0.9345	2.29	0.0915	2.98	0.0570	1.23	0.3309	2.84	0.0516	1.04	0.4089	1.08	0.3925

Table A.4. ANOVA for composition of plant parts of Napier grass cultivars collected across the three elevations and two harvest seasons (within a year for two years, thus 4 harvest seasons)

Source	DF	DF Den	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	2	6	4.92	0.0536	1.86	0.2350	0.23	0.8017	6.89	0.0274	18.37	0.0027	10.32	0.0113	0.77	0.5059
Cultivar	1	6	0.47	0.5196	6.45	0.0439	25.40	0.0023	7.20	0.0365	4.77	0.0752	29.14	0.0016	0.06	0.8151
Cultivar*Elevation	2	6	0.09	0.9178	0.50	0.6296	5.40	0.0452	2.16	0.1963	0.54	0.6083	5.49	0.0438	1.10	0.3923
Plant part	1	12	0.93	0.3549	421.64	<.0001	817.67	<.0001	1390.27	<.0001	1109.86	<.0001	1189.00	<.0001	1336.61	<.0001
Plant part*Elevation	2	12	82.77	<.0001	59.21	<.0001	42.81	<.0001	17.29	0.0003	9.56	0.0034	36.68	<.0001	0.05	0.9528
Plant part*Cultivar	1	12	0.76	0.4003	0.11	0.7483	1.06	0.3239	0.09	0.7703	0.88	0.3663	0.00	0.9929	0.63	0.4417
Plant part*Elevation*Cultivar	2	12	3.53	0.0637	12.86	0.0013	8.68	0.0050	2.99	0.0882	1.00	0.3970	2.79	0.1010	2.60	0.1149
Harvest season	3	72	3.74	0.0149	11.69	<.0001	30.75	<.0001	36.39	<.0001	2.94	0.0387	38.86	<.0001	22.43	<.0001
Harvest season*Elevation	6	72	15.43	<.0001	12.97	<.0001	6.30	<.0001	10.53	<.0001	5.40	0.0001	11.53	<.0001	8.29	<.0001

Table A.4. (Continued) ANOVA for composition of plant parts of Napier grass cultivars collected across the three elevations and two harvest seasons (within a year for two years, thus 4 harvest seasons)

Source	DF	DF Den	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Harvest season*Cultivar	3	72	3.47	0.0205	0.68	0.5657	1.98	0.1244	0.07	0.9776	2.60	0.0585	0.42	0.7392	1.36	0.2607
Harvest season*Plant part	3	72	0.39	0.7623	2.36	0.0788	4.42	0.0066	6.33	0.0007	2.30	0.0846	3.36	0.0233	13.46	<.0001
Harvest season*Elevation*Cultivar	6	72	1.42	0.2184	1.92	0.0887	0.90	0.5004	0.57	0.7510	0.11	0.9952	0.78	0.5921	0.49	0.8142
Harvest season*Elevation *Plant part	6	72	6.45	<.0001	5.09	0.0002	2.77	0.0176	2.22	0.0513	2.40	0.0362	2.07	0.0672	3.33	0.0061
Harvest season*Cultivar*Plant part	3	72	0.07	0.9735	0.49	0.6930	0.68	0.5666	0.84	0.4746	1.04	0.3815	0.94	0.4247	0.54	0.6555
Harvest season*Elevation *Cultivar*Plant part	6	72	1.59	0.1616	1.67	0.1402	0.83	0.5506	0.45	0.8405	0.78	0.5896	0.71	0.6411	0.39	0.8811

Table A.5. ANOVA for composition of plant parts of Napier grass cultivars collected across the three elevations and two harvest seasons (within a year for two years, thus 4 harvests)

Source	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	4.76	0.0573	1.65	0.2688	0.25	0.7872	6.56	0.0304	18.38	0.0027	10.33	0.0113	0.60	0.5783
Cultivar	0.51	0.5038	6.73	0.0408	27.06	0.0019	7.11	0.0376	4.40	0.0839	28.94	0.0017	0.01	0.9162
Cultivar*Elevation	0.09	0.9185	0.49	0.6369	5.53	0.0428	1.94	0.2251	0.52	0.6229	5.32	0.0468	1.14	0.382
Plant part	1.04	0.329	477.04	<.0001	767.98	<.0001	1352.45	<.0001	1142.68	<.0001	1180.13	<.0001	1299.61	<.0001
Plant part *Elevation	86.87	<.0001	66.70	<.0001	39.28	<.0001	16.41	0.0004	10.12	0.0027	36.25	<.0001	0.02	0.9836
Plant part*Cultivar	0.74	0.4064	0.05	0.8191	1.16	0.3043	0.15	0.7091	0.82	0.3837	0.00	0.991	0.94	0.3521
Plant part* Elevation*Cultivar	3.58	0.0612	13.88	0.0009	8.08	0.0065	2.90	0.0937	1.06	0.3776	2.76	0.1032	2.65	0.1113
Season	6.71	0.0111	21.50	<.0001	63.77	<.0001	82.56	<.0001	4.98	0.028	87.19	<.0001	35.56	<.0001
Season*Elevation	40.74	<.0001	18.64	<.0001	4.11	0.0193	10.01	0.0001	5.62	0.0049	12.99	<.0001	2.12	0.1253
Season*Cultivar	7.02	0.0095	0.04	0.8391	3.60	0.0609	0.02	0.8907	4.61	0.0344	0.81	0.3691	1.55	0.2165
Season*Plant part	0.59	0.4438	2.61	0.1092	7.55	0.0072	13.60	0.0004	2.24	0.1382	7.10	0.0091	19.29	<.0001
Season*Elevation* Cultivar	2.78	0.0673	2.07	0.1323	0.69	0.5028	0.77	0.4677	0.02	0.9776	0.85	0.4296	0.70	0.4989

Table A.5. (Continued) ANOVA for composition of plant parts of Napier grass cultivars collected across the three elevations and two harvest seasons (within a year for two years, thus 4 harvests)

Source	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Season*Elevation*Plant part	17.31	<.0001	6.98	0.0015	2.54	0.0844	2.51	0.0867	0.40	0.6709	1.64	0.199	2.72	0.0712
Season*Cultivar*Plant part	0.01	0.9395	0.41	0.5226	0.59	0.4435	2.22	0.1396	1.08	0.3008	2.16	0.1446	1.16	0.2836
Season*Elevation*Cultivar*Plant part	4.56	0.0129	3.19	0.0456	0.42	0.6581	0.07	0.9335	0.91	0.4076	0.02	0.9813	0.17	0.8439

Table A.6. ANOVA for composition of Napier grass cultivars collected across the three elevations and two harvest seasons (within a year for two years, thus 4 harvests)

Source	DF	DF_{Den}	Ash		Ash free extractives		NDF		ADF		ADL		Hemicellulose		Cellulose	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	2	6	5.32	0.0468	0.16	0.8593	2.10	0.2031	14.32	0.0052	3.09	0.1198	17.35	0.0032	18.48	0.0027
Cultivar	1	6	0.44	0.5331	5.05	0.0658	15.18	0.0080	2.44	0.1690	0.12	0.7363	8.95	0.0242	10.14	0.0190
Cultivar*Elevation	2	6	0.09	0.9120	0.91	0.4507	5.54	0.0433	1.59	0.2783	0.93	0.4455	1.60	0.2773	3.91	0.0820
Harvest by season	3	36	5.55	0.0031	14.50	<.0001	29.64	<.0001	24.49	<.0001	19.33	<.0001	2.01	0.1293	26.95	<.0001
Harvest by season*Elevation	6	36	14.62	<.0001	13.40	<.0001	7.12	<.0001	10.92	<.0001	11.60	<.0001	5.22	0.0006	10.41	<.0001
Harvest by season*Cultivar	3	36	3.72	0.0199	0.27	0.8498	1.55	0.2193	0.51	0.6779	1.87	0.1529	2.76	0.0560	0.43	0.7302
Harvest by season*Elevation*Cultivar	6	36	1.85	0.1175	1.97	0.0958	0.71	0.6411	0.25	0.9580	0.43	0.8508	0.21	0.9704	0.34	0.9109

Table A.7. ANOVA for composition of Napier grass cultivars collected across the three elevations and two harvest years

Source	DF	DF Den	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	2	6	2.26	0.1834	0.14	0.8732	2.19	0.1917	28.32	0.0007	20.89	0.0024	49.48	0.0002	2.24	0.1874
Cultivar	1	6	0.01	0.9112	5.01	0.0656	30.31	0.0014	3.46	0.1132	4.74	0.0777	11.71	0.0157	0.06	0.8122
Cultivar*Elevation	2	6	0.10	0.9090	0.82	0.4837	7.28	0.0239	0.65	0.5572	1.31	0.3435	1.61	0.2809	0.73	0.5189
Harvest year	1	12	0.68	0.4277	3.10	0.1049	5.01	0.0449	2.32	0.1538	0.81	0.3852	0.02	0.8934	13.29	0.0037
Harvest year*Elevation	2	12	5.76	0.0184	5.93	0.0170	8.48	0.0050	18.08	0.0003	1.42	0.2799	14.25	0.0007	18.35	0.0003
Harvest year*Cultivar	1	12	3.63	0.0818	0.04	0.8502	0.90	0.3620	0.29	0.6021	2.95	0.1112	0.06	0.8105	3.21	0.1001
Harvest year*Elevation*Cultivar	2	12	0.13	0.8764	0.43	0.6631	0.25	0.7807	0.05	0.9487	0.27	0.7660	0.27	0.7684	0.16	0.8505

Table A.8. ANOVA for composition of Energycane and Napier grass collected across the three elevations and two harvest years

Source	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	28.44	0.0008	19.96	0.0021	13.02	0.0062	2.82	0.1345	19.83	0.0024	2.33	0.1761	9.44	0.0132
Crop type	95.64	0.0001	1949.88	<.0001	881.49	<.0001	467.71	<.0001	178.63	<.0001	591.31	<.0001	106.77	<.0001
Crop type*Elevation	7.39	0.0229	67.22	0.0001	29.09	0.0008	25.12	0.0011	0.81	0.4866	26.11	0.0010	12.58	0.0070
Harvest year	3.51	0.0671	0.41	0.5237	0.05	0.8255	0.08	0.7821	0.00	0.9866	0.19	0.6655	5.47	0.0236
Harvest year*Elevation	3.96	0.0257	1.75	0.1853	2.30	0.1112	4.10	0.0227	5.44	0.0074	1.98	0.1495	12.43	<.0001
Harvest year*Crop type	1.19	0.2808	2.43	0.1257	2.18	0.1462	1.12	0.2942	1.50	0.2272	0.41	0.5251	3.81	0.0569
Harvest year* Elevation*Crop type	2.60	0.0848	6.42	0.0034	6.27	0.0038	6.38	0.0035	0.86	0.4312	5.98	0.0048	3.72	0.0316

BIOMASS COMPOSITION FOR 2015 HARVESTS USED FOR ANAEROBIC DIGESTION STUDIES

Table A.9. ANOVA for composition of plant parts of Energycane cultivars collected in 2015 across the three elevations

Source	DF	DF Den	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	2	6	60.70	0.0001	11.55	0.0088	23.33	0.0015	3.76	0.0874	25.94	0.0011	0.96	0.4341	14.49	0.0050
Cultivar	1	6	30.37	0.0015	6.93	0.0389	0.35	0.5771	0.07	0.8019	1.56	0.2585	0.03	0.8676	0.37	0.5669
Cultivar*Elevation	2	6	1.73	0.2555	1.02	0.4159	0.60	0.5765	0.60	0.5792	0.76	0.5079	0.44	0.6606	2.11	0.2027
Plant part	3	36	443.40	<.0001	893.59	<.0001	722.18	<.0001	267.76	<.0001	359.17	<.0001	320.77	<.0001	120.37	<.0001
Plant part*Elevation	6	36	28.72	<.0001	4.49	0.0017	16.11	<.0001	14.08	<.0001	6.77	0.0001	12.63	<.0001	7.97	<.0001
Plant part*Cultivar	3	36	2.01	0.1292	1.61	0.2033	3.71	0.0201	4.55	0.0084	1.11	0.3565	4.18	0.0122	2.33	0.0910
Plant part* Elevation*Cultivar	6	36	0.70	0.6543	0.17	0.9826	0.38	0.8895	0.88	0.5215	0.66	0.6821	1.12	0.3687	1.32	0.2746

Table A.10. ANOVA for composition of Energycane cultivars collected in 2015 across the three elevations

Source	DF	DF Den	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	2	6	8.96	0.0158	10.75	0.0104	8.46	0.0179	5.53	0.0435	1.92	0.2263	3.09	0.1195	16.07	0.0039
Cultivar	1	6	6.95	0.0387	3.39	0.1153	1.15	0.3244	0.13	0.7324	1.05	0.3446	0.21	0.6600	0.06	0.8170
Cultivar*Elevation	2	6	2.10	0.2032	1.78	0.2471	1.00	0.4215	1.15	0.3772	0.44	0.6643	1.32	0.3342	1.27	0.3468

Table A.11. ANOVA for composition of plant parts of Napier grass cultivars collected in two seasons of 2015 across the three elevations

Source	DF	DF Den	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	2	6	2.65	0.1495	0.70	0.5330	2.97	0.1268	1.48	0.2994	2.15	0.1973	0.25	0.7876	4.14	0.0741
Cultivar	1	6	1.72	0.2382	5.29	0.0610	7.18	0.0366	5.47	0.0579	1.49	0.2683	13.65	0.0102	0.34	0.5796
Cultivar*Elevation	2	6	0.40	0.6867	0.04	0.9614	1.17	0.3725	0.89	0.4600	0.46	0.6541	1.60	0.2769	1.12	0.3849
Plant part	1	12	2.47	0.1419	401.87	<.0001	305.97	<.0001	785.08	<.0001	385.07	<.0001	465.46	<.0001	1705.71	<.0001
Plant part* Elevation	2	12	107.01	<.0001	50.14	<.0001	12.24	0.0013	11.63	0.0016	3.94	0.0485	21.92	0.0001	1.10	0.3644
Plant part*Cultivar	1	12	0.67	0.4281	1.00	0.3363	0.03	0.8747	0.04	0.8376	0.40	0.5393	0.03	0.8748	1.56	0.2348
Plant part* Elevation *Cultivar	2	12	3.81	0.0524	8.71	0.0046	1.40	0.2847	1.30	0.3086	0.29	0.7568	1.08	0.3698	3.58	0.0602
Season	1	24	7.36	0.0121	15.44	0.0006	109.45	<.0001	80.43	<.0001	4.26	0.0499	101.46	<.0001	24.58	<.0001
Season*Elevation	2	24	19.38	<.0001	35.67	<.0001	7.97	0.0022	17.92	<.0001	12.66	0.0002	16.16	<.0001	13.23	0.0001
Season*Cultivar	1	24	0.51	0.4819	1.26	0.2732	8.35	0.0081	0.00	0.9903	4.98	0.0353	0.59	0.4501	1.71	0.2035
Season*Plant part	1	24	0.27	0.6113	4.64	0.0414	17.16	0.0004	7.87	0.0098	0.00	0.9804	4.28	0.0496	11.24	0.0026
Season*Elevation *Cultivar	2	24	1.71	0.2016	3.73	0.0387	0.84	0.4454	0.30	0.7411	0.02	0.9808	1.25	0.3051	0.35	0.7049

Table A.11. (Continued) ANOVA for composition of plant parts of Napier grass cultivars collected in two seasons of 2015 across the three elevations

Source	DF	DF Den	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Season*Elevation* Plant part	2	24	3.59	0.0431	2.94	0.0722	14.01	0.0001	7.82	0.0024	4.59	0.0204	6.07	0.0074	7.41	0.0031
Season*Cultivar*Plant part	1	24	0.08	0.7749	0.00	0.9722	0.18	0.6714	1.66	0.2105	1.26	0.2727	1.47	0.2369	1.23	0.2790
Season*Elevation* Cultivar *Plant part	2	24	2.15	0.1388	2.90	0.0742	2.04	0.1519	0.45	0.6411	0.68	0.5171	0.15	0.8656	1.14	0.3351

Table A.12. ANOVA for composition of Napier grass cultivars collected in 2015 across the three elevations

Source	DF	DF Den	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	2	6	7.37	0.0242	2.02	0.2130	3.24	0.1109	17.71	0.0030	3.12	0.1177	25.76	0.0011	7.76	0.0217
Cultivar	1	6	1.06	0.3424	4.22	0.0859	6.84	0.0398	2.91	0.1387	0.17	0.6905	5.69	0.0543	0.35	0.5780
Cultivar*Elevation	2	6	0.27	0.7693	0.43	0.6691	1.45	0.3063	0.37	0.7047	0.35	0.7159	0.96	0.4352	0.85	0.4716

Table A.13. ANOVA for composition of Energycane and Napier grass collected in 2015 across the three elevations

Source	DF	DF_{Den}	Ash		Ash free extractives		NDF		ADF		Hemicellulose		Cellulose		Lignin (ADL)	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	2	6	14.14	0.0054	6.27	0.0339	1.48	0.3008	0.61	0.5739	2.46	0.1659	0.51	0.6219	9.74	0.0131
Crop type	1	6	93.97	0.0001	1176.59	<.0001	730.25	<.0001	583.51	<.0001	149.13	<.0001	612.22	<.0001	149.42	<.0001
Crop type* Elevation	2	6	0.99	0.4261	12.41	0.0074	12.16	0.0077	15.43	0.0043	2.95	0.1283	11.80	0.0083	11.11	0.0096

ANAEROBIC DIGESTION FOR BIOGAS PRODUCTION

Table A.14. ANOVA for methane and biogas yields from plant parts of Energycane cultivars collected in 2015 across the three elevations

Source	DF	DF_{Den}	Methane content		Specific methane yield		Total methane yield	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	2	6	4.02	0.0782	1.16	0.3744	8.58	0.0174
Cultivar	1	6	4.72	0.0728	3.90	0.0957	0.34	0.5824
Cultivar*Elevation	2	6	6.00	0.0370	3.17	0.1148	1.02	0.4171
Plant part	3	36	148.98	<.0001	103.58	<.0001	171.47	<.0001
Plant part*Elevation	6	36	4.61	0.0014	5.66	0.0003	8.58	<.0001
Plant part*Cultivar	3	36	7.75	0.0004	0.88	0.4613	0.10	0.9614
Plant part*Elevation *Cultivar	6	36	4.17	0.0028	2.21	0.0643	1.08	0.3915

Table A.15. ANOVA for methane and biogas yields from Energycane cultivars collected in 2015 across the three elevations

Source	DF	DF_{Den}	Methane content		Specific methane yield		Total methane yield	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	2	6	1.49	0.2990	7.18	0.0256	8.58	0.0174
Cultivar	1	6	0.30	0.6062	1.43	0.2765	0.34	0.5824
Cultivar*Elevation	2	6	0.71	0.5280	1.03	0.4121	1.02	0.4171

Table A.16. ANOVA for methane and biogas yields from plant parts of Napier grass cultivars collected in two seasons of 2015 across the three elevations

Source	DF	DF_{Den}	Methane content		Specific methane yield		Methane yield	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	2	6	13.25	0.0063	0.18	0.8370	37.78	0.0004
Cultivar	1	6	3.58	0.1074	1.12	0.3316	1.30	0.2980
Cultivar*Elevation	2	6	2.46	0.1657	10.31	0.0115	2.80	0.1387
Plant part	1	12	17.21	0.0014	959.17	<.0001	107.96	<.0001
Plant part*Elevation	2	12	2.55	0.1190	0.10	0.9076	52.54	<.0001
Plant part*Cultivar	1	12	7.95	0.0155	6.60	0.0246	0.67	0.4307
Plant part*Elevation*Cultivar	2	12	8.86	0.0043	5.81	0.0172	6.89	0.0102
Season	1	24	1.12	0.3003	28.48	<.0001	29.14	<.0001
Season*Elevation	2	24	5.18	0.0135	0.42	0.6636	21.71	<.0001
Season*Cultivar	1	24	0.18	0.6746	0.24	0.6266	1.19	0.2856
Season*Plant part	1	24	5.61	0.0263	0.10	0.7495	1.67	0.2084
Season*Elevation*Cultivar	2	24	0.34	0.7121	6.73	0.0048	1.15	0.3323
Season*Elevation*Plant part	2	24	0.30	0.7425	0.46	0.6370	5.17	0.0135
Season*Cultivar*Plant part	1	24	0.55	0.4660	2.14	0.1568	0.08	0.7755
Season*Elevation*Cultivar*Plant part	2	24	1.17	0.3268	1.06	0.3615	0.43	0.6562

Table A.17. ANOVA for methane and biogas yields from Napier grass cultivars collected in 2015 across the three elevations

Source	DF	DF_{Den}	Methane content		Specific methane yield		Total methane yield	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	2	6	4.86	0.0556	1.26	0.3483	37.78	0.0004
Cultivar	1	6	5.76	0.0533	0.00	0.9824	1.30	0.2981
Cultivar*Elevation	2	6	3.69	0.0902	7.11	0.0261	2.80	0.1387

Table A.18. ANOVA for methane and biogas yields from Energycane and Napier grass collected in 2015 across the three elevations

Source	DF	DF_{Den}	Methane content		Specific methane yield		Total methane yield	
			F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
Elevation	2	6	10.00	0.0123	1.54	0.2893	20.23	0.0022
Crop type	1	6	95.91	0.0001	144.84	<.0001	29.33	0.0016
Crop type* Elevation	2	6	0.57	0.5958	4.47	0.0647	2.15	0.1975

APPENDIX B: COMPOSITIONAL DATA OF ENERGY CROPS

Table B.1. Least square means of composition of different plant parts of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2013	30	77-9271	Leaf, Bottom	10.03 ± 0.74 abcdefghijkq	18.61 ± 1.49 rstuvwxy	71.36 ± 1.31 bcdefgh	47.10 ± 1.11 abcdefg	24.26 ± 0.84 fghijklmno	39.31 ± 0.93 abcdegh	7.78 ± 0.35 abc
			Leaf, Top	7.61 ± 0.74 efghijklmnoprsxy	23.01 ± 1.49 klmnopqrstuvwxyz	69.38 ± 1.31 cdefghijkl	41.33 ± 1.11 fghijklmnopqrstuvxz	28.05 ± 0.84 bcdefghijk	36.60 ± 0.93 defghijklmnopqw	4.73 ± 0.35 jklmnopqr
			Stem, Bottom	2.15 ± 0.90 uvw	48.82 ± 1.83 abcd	48.92 ± 1.58 wxyza1	37.24 ± 1.34 rstuvwxyza1b1c1d1e1f1	11.76 ± 1.03 r	30.00 ± 1.12 rtuvxyza1b1c1	7.24 ± 0.43 abcdefgh
			Stem, Top	6.23 ± 0.74 hijklmnoprstuxy	26.93 ± 1.49 hijklmnopqrz	66.83 ± 1.31 defghijklmnopq	41.80 ± 1.11 cdefghijklmnopqrstuxz	25.03 ± 0.84 efghijklmno	35.78 ± 0.93 efghijklmnopqsw	6.02 ± 0.35 bcdefghijklmnopqr
		MOL 6136	Leaf, Bottom	11.93 ± 0.74 abcdeq	18.23 ± 1.49 rstuvwxy	69.84 ± 1.31 cdefghijkl	47.9 ± 1.11 abcde	21.94 ± 0.84 nop	42.42 ± 0.93 ab	5.47 ± 0.35 efghijklmnopqr
			Leaf, Top	10.62 ± 0.74 abcdefghq	20.13 ± 1.49 qrstuvwxyz	69.25 ± 1.31 cdefghijklm	41.98 ± 1.11 cdefghijklmnopqrstxz	27.28 ± 0.84 bcdefghijklm	36.44 ± 0.93 defghijklmnopqw	5.53 ± 0.35 defghijklmnopqr
			Stem, Bottom	5.22 ± 0.90 jklmnoprstuvwxy	39.66 ± 1.83 cdef	54.83 ± 1.58 stuvwxy	39.69 ± 1.34 hijklmnopqrstuvwxyza1b1c1	15.37 ± 1.03 rs	32.14 ± 1.12 opqrstuvxyzb1c1	7.62 ± 0.43 abcdef
			Stem, Top	9.28 ± 0.74 abcdefghijklmnqx	26.38 ± 1.49 hijklmnopqrsz	64.34 ± 1.31 ghijklmnopqr	41.88 ± 1.11 cdefghijklmnopqrstuxz	22.46 ± 0.84 mno	36.62 ± 0.93 cdefghijklmnopw	5.26 ± 0.35 ghijklmnopqr

Table B.1. (Continued) Least square means of composition of different plant parts of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2013	305	77-9271	Leaf, Bottom	4.44 ± 0.74 oprstuvwy	21.50 ± 1.49 pqrstuvwxyz	74.06 ± 1.31 abcde	44.73 ± 1.11 abcdefghijklmnox	29.34 ± 0.84 abcdefg	38.44 ± 0.93 abcdefghijknw	6.29 ± 0.35 abcdefghijklmnop
			Leaf, Top	4.02 ± 0.74 prstuvw	23.07 ± 1.49 nopqrstuvwxyz	72.91 ± 1.31 abcdef	40.74 ± 1.11 ghijklmnopqrstuvwxz	32.17 ± 0.84 ab	35.10 ± 0.93 efghijklmnopqrsw	5.64 ± 0.35 defghijklmnopqr
			Stem, Bottom	1.15 ± 0.74 w	50.84 ± 1.49 ab	48.01 ± 1.31 xyza1	32.25 ± 1.11 b1d1e1f1g1	15.77 ± 0.84 r	25.89 ± 0.93 za1d1	6.36 ± 0.35 abcdefghijklmnop
			Stem, Top	3.67 ± 0.74 rstuvw	31.69 ± 1.49 fghijklm	64.64 ± 1.31 hijklmnopq	39.76 ± 1.11 hijklmnopqrstuvwxyz	24.88 ± 0.84 efghijklmno	33.24 ± 0.93 lmopqrstuvyb1	6.52 ± 0.35 abcdefghijklmn
		MOL 6136	Leaf, Bottom	6.38 ± 0.74 ghijklmnoprstuxy	22.07 ± 1.49 pqrstuvwxyz	71.55 ± 1.31 bcdefgh	43.78 ± 1.11 cdefghijklmnopqrx	27.77 ± 0.84 bcdefghijkl	38.18 ± 0.93 abcdefghijklnow	5.59 ± 0.35 defghijklmnopqr
			Leaf, Top	5.25 ± 0.74 mnoprstuvwxy	23.30 ± 1.49 lnopqrstuvwxyz	71.45 ± 1.31 bcdefgh	38.25 ± 1.11 pqrstuvwyza1b1c1	33.20 ± 0.84 a	33.31 ± 0.93 jklmnopqrstuvyb1	4.94 ± 0.35 hijklmnopqr
			Stem, Bottom	2.66 ± 0.9 stuvw	51.83 ± 1.83 ab	45.37 ± 1.58 yza1	29.57 ± 1.34 f1g1	15.84 ± 1.03 qrs	24.86 ± 1.12 a1d1	4.68 ± 0.43 hijklmnopqr
			Stem, Top	5.28 ± 0.74 mnoprstuvwxy	32.08 ± 1.49 efghijkm	62.64 ± 1.31 lmnopqrst	35.84 ± 1.11 tuvwya1b1c1d1e1f1	26.79 ± 0.84 defghijklmn	30.30 ± 0.93 stuvxyza1b1c1	5.54 ± 0.35 efghijklmnopqr

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the p ≤ 0.05 level

Table B.1. (Continued) Least square means of composition of different plant parts of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2013	915	77-9271	Leaf, Bottom	13.71 ± 0.74 ac	18.19 ± 1.49 rstuvwxy	68.10 ± 1.31 defghijklmn	41.10 ± 1.11 eghijklmnopqrstuvwxz	26.99 ± 0.84 cdefghijklmn	35.62 ± 0.93 efghijklmnopqrstw	5.48 ± 0.35 efghijklmnopqr
			Leaf, Top	7.72 ± 0.74 efghijklmnoprxy	25.01 ± 1.49 jklmnopqrstuvz	67.27 ± 1.31 defghijklmnop	39.64 ± 1.11 hijklmnopqrstuvwxz	27.64 ± 0.84 bcdefghijkl	34.85 ± 0.93 efghijklmnopqrstuw	4.78 ± 0.35 ijklmnopqr
			Stem, Bottom	2.80 ± 0.74 tuvw	48.83 ± 1.49 abc	48.37 ± 1.31 xyz	32.19 ± 1.11 c1d1e1f1g1	16.18 ± 0.84 qrs	26.85 ± 0.93 za1d1	5.35 ± 0.35 fghijklmnopqr
			Stem, Top	6.86 ± 0.74 fghijklmnoprstuxy	34.03 ± 1.49 efghi	59.11 ± 1.31 qrstuv	38.04 ± 1.11 nqrstuvwxyza1b1c1	21.07 ± 0.84 opqs	33.46 ± 0.93 jklmnopqrstuvxb1	4.59 ± 0.35 mnopqr
		MOL 6136	Leaf, Bottom	13.54 ± 0.74 ab	21.12 ± 1.49 qrstuvwxyz	65.34 ± 1.31 fghijklmnopq	39.85 ± 1.11 hijklmnopqrstuvwxz	25.50 ± 0.84 efghijklmno	34.42 ± 0.93 efghijklmnopqrstuw	5.43 ± 0.35 fghijklmnopqr
			Leaf, Top	8.87 ± 0.90 abcdefghijklmnopqxy	25.67 ± 1.83 ghijklmnopqrstuvwxz	65.58 ± 1.58 efghijklmnopqr	37.86 ± 1.34 mnopqrstuvwxyza1b1c1d1	27.66 ± 1.03 abcdefghijklmn	32.98 ± 1.12 jklmnopqrstuvwxyb1	4.77 ± 0.43 hijklmnopqr
			Stem, Bottom	4.68 ± 0.90 noprstuvwxy	49.77 ± 1.83 abc	45.44 ± 1.58 yza1	30.7 ± 1.34 d1e1f1g1	14.8 ± 1.03 r	26.61 ± 1.12 za1c1d1	4.11 ± 0.43 opqr
			Stem, Top	9.42 ± 0.74 abcdefghijklmnqx	27.87 ± 1.49 ghijklmnopqz	62.70 ± 1.31 kmnopqrst	39.18 ± 1.11 lmnopqrstuvwxyz	23.53 ± 0.84 klmno	34.25 ± 0.93 efghijklmnopqrstuw	4.93 ± 0.35 hijklmnopqr

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the p ≤ 0.05 level

Table B.1. (Continued) Least square means of composition of different plant parts of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2014	30	77-9271	Leaf, Bottom	10.77 ± 0.74 abcdefgq	18.76 ± 1.49 rstuvwxy	70.47 ± 1.31 bcdefghijkl	46.25 ± 1.11 abcdefghj	24.22 ± 0.84 ghijklmno	38.99 ± 0.93 abcdefghjw	7.27 ± 0.35 abcdefg
			Leaf, Top	7.73 ± 0.74 efghijklmnoprxy	20.48 ± 1.49 qrstuvwxyz	71.80 ± 1.31 abcdefgh	42.95 ± 1.11 cdefghijklmnopqrsx	28.85 ± 0.84 abcdefghi	36.68 ± 0.93 cdefghijklmnopqw	6.27 ± 0.35 abcdefghijklmnop
			Stem, Bottom	1.52 ± 0.74 vw	50.45 ± 1.49 ab	48.03 ± 1.31 xyza1	34.69 ± 1.11 wya1b1c1d1e1f1	13.35 ± 0.84 r	27.95 ± 0.93 xyza1b1c1d1	6.74 ± 0.35 abcdefghijkl
			Stem, Top	6.15 ± 0.74 hijklmnoprstuxy	34.01 ± 1.49 efghij	59.84 ± 1.31 pqrstu	37.52 ± 1.11 rstuvwyza1b1c1d1	22.33 ± 0.84 mno	32.80 ± 0.93 nopqrstuvxyb1c1	4.72 ± 0.35 jklmnopqr
		MOL 6136	Leaf, Bottom	13.41 ± 0.74 abcq	18.73 ± 1.49 rstuvwxy	67.87 ± 1.31 defghijklmo	44.19 ± 1.11 abcdefghijklmnopqx	23.67 ± 0.84 jklmno	37.44 ± 0.93 abcdefghijklmnow	6.75 ± 0.35 abcdefghijkl
			Leaf, Top	9.61 ± 0.74 abcdefghijklmqx	22.98 ± 1.49 klmnopqrstuvwxyz	67.41 ± 1.31 defghijklmnop	39.77 ± 1.11 iklmnopqrstuvwxyz	27.64 ± 0.84 bcdefghijkl	33.98 ± 0.93 fijklmnopqrstuvw	5.79 ± 0.35 bcdefghijklmnopqr
			Stem, Bottom	3.80 ± 0.74 rstuvw	35.85 ± 1.49 efg	60.35 ± 1.31 npqrstu	43.35 ± 1.11 bcdefghijklmnopqrsx	17.00 ± 0.84 pqrs	35.15 ± 0.93 efghijklmnopqrstw	8.20 ± 0.35 a
			Stem, Top	9.39 ± 0.74 abcdefghijklmnqx	26.39 ± 1.49 hijklmnopqrsz	64.22 ± 1.31 hijklmnopqr	41.25 ± 1.11 ghijklmnopqrstuvxz	22.98 ± 0.84 lmno	35.65 ± 0.93 efghijklmnopqrstw	5.60 ± 0.35 defghijklmnopqr

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.1. (Continued) Least square means of composition of different plant parts of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2014	305	77-9271	Leaf, Bottom	5.64 ± 0.74 klmnoprstuvxy	22.02 ± 1.49 pqrstuvwxyz	72.34 ± 1.31 abcdef	44.5 ± 1.11 abcdefghijklmnopqx	27.84 ± 0.84 bcdefghijkl	36.87 ± 0.93 bdefghijklmnopqw	7.63 ± 0.35 abcd
			Leaf, Top	11.55 ± 0.74 abcdeq	17.13 ± 1.49 tuvwxy	71.32 ± 1.31 bcdefgh	41.67 ± 1.11 cdefghijklmnopqrstxz	29.65 ± 0.84 abcde	35.53 ± 0.93 efghijklmnopqrsw	6.14 ± 0.35 abcdefghijklmnopq
			Stem, Bottom	7.51 ± 0.74 efghijklmnoprsxy	38.80 ± 1.49 def	53.69 ± 1.31 uvwxy	36.86 ± 1.11 stuvwyza1b1c1d1e1	16.83 ± 0.84 pqrs	30.04 ± 0.93 tuvxyza1b1c1	6.81 ± 0.35 abcdefghijk
			Stem, Top	11.29 ± 0.74 abcdefq	25.41 ± 1.49 ijklmnopqrstz	63.3 ± 1.31 iklmnopqrs	39.4 ± 1.11 kmnopqrstuvwxyz	23.9 ± 0.84 hijklmno	34.18 ± 0.93 efghijklmnopqrstuw	5.22 ± 0.35 ghijklmnopqr
		MOL 6136	Leaf, Bottom	9.09 ± 0.74 bdefghijklmnoqx	17.23 ± 1.49 tuvwxy	73.68 ± 1.31 abcde	45.90 ± 1.11 abcdefghil	27.78 ± 0.84 bcdefghijkl	39.26 ± 0.93 abcdefhiw	6.64 ± 0.35 abcdefghijklm
			Leaf, Top	12.86 ± 0.74 abcdq	16.19 ± 1.49 vwxy	70.94 ± 1.31 bcdefghj	41.30 ± 1.11 eghijklmnopqrstwxz	29.65 ± 0.84 abcde	35.22 ± 0.93 efghijklmnopqrstw	6.08 ± 0.35 bcdefghijklmnopqr
			Stem, Bottom	7.64 ± 0.74 efghijklmnoprxy	35.49 ± 1.49 efgh	56.87 ± 1.31 rstuvw	39.71 ± 1.11 jkmnopqrstuvwxyz	17.17 ± 0.84 pqrs	32.88 ± 0.93 mpqrstuvxyb1c1	6.83 ± 0.35 abcdefghij
			Stem, Top	10.53 ± 0.74 abcdefghiq	23.43 ± 1.49 klmnopqrstuvwxyz	66.04 ± 1.31 fghijklmnopq	42.27 ± 1.11 cdefghijklmnopqrsxz	23.77 ± 0.84 ijklmno	36.54 ± 0.93 defghijklmnopqw	5.73 ± 0.35 cdefghijklmnopqr

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the p ≤ 0.05 level

Table B.1. (Continued) Least square means of composition of different plant parts of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2014	915	77-9271	Leaf, Bottom	8.06 ± 0.74 efghijklmnoprxy	15.58 ± 1.49 y	76.36 ± 1.31 abc	48.31 ± 1.11 abc	28.05 ± 0.84 bcdefghijkl	41.67 ± 0.93 abcd	6.64 ± 0.35 abcdefghijklm
			Leaf, Top	6.21 ± 0.74 hijklmnoprstuxy	23.22 ± 1.49 mopqrstuvwxyz	70.57 ± 1.31 bcdefghijl	41.60 ± 1.11 eghijklmnopqrstuvxz	28.96 ± 0.84 abcdefgh	36.11 ± 0.93 efghijklmnopqrw	5.49 ± 0.35 efghijklmnopqr
			Stem, Bottom	1.59 ± 0.74 vw	54.08 ± 1.49 ab	44.33 ± 1.31 za1	30.51 ± 1.11 e1f1g1	13.81 ± 0.84 r	25.50 ± 0.93 a1d1	5.01 ± 0.35 hijklmnopqr
			Stem, Top	6.07 ± 0.74 ijklmnoprstuxy	31.19 ± 1.49 fghijklmno	62.74 ± 1.31 kmnopqrst	37.78 ± 1.11 qrstuvwyza1b1c1d1	24.96 ± 0.84 efghijklmno	33.28 ± 0.93 klmnopqrstuvxb1	4.49 ± 0.35 nopqr
		MOL 6136	Leaf, Bottom	9.94 ± 0.74 abcdefghijkq	16.01 ± 1.49 wxy	74.05 ± 1.31 abcd	48.1 ± 1.11 abcdf	25.95 ± 0.84 defghijklmno	42.47 ± 0.93 ab	5.63 ± 0.35 defghijklmnopqr
			Leaf, Top	7.50 ± 0.74 efghijklmnoprstxy	25.37 ± 1.49 ijklmnopqrstuz	67.13 ± 1.31 defghijklmnop	38.57 ± 1.11 mnopqrstuvwxyza1b1	28.57 ± 0.84 abcdefghij	33.67 ± 0.93 hjklmnopqrstuvw	4.90 ± 0.35 hijklmnopqr
			Stem, Bottom	2.51 ± 0.74 uvw	57.28 ± 1.49 a	40.21 ± 1.31 a1	26.84 ± 1.11 g1	13.37 ± 0.84 r	22.58 ± 0.93 d1	4.25 ± 0.35 pqr
			Stem, Top	5.49 ± 0.74 lmnoprstuvwxy	35.31 ± 1.49 efgh	59.20 ± 1.31 qrstuv	35.50 ± 1.11 tuvwyza1b1c1d1e1f1	23.69 ± 0.84 jklmno	31.20 ± 0.93 pqrstuvxyzb1c1	4.30 ± 0.35 pqr

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.1. (Continued) Least square means of composition of different plant parts of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2015	30	77-9271	Leaf, Bottom	10.33 ± 0.74 abcdefghijq	19.02 ± 1.49 rstuvwxyz	70.66 ± 1.31 bcdefghik	44.48 ± 1.11 abcdefghijklmnopq	26.18 ± 0.84 defghijklmno	38.19 ± 0.93 abcdefghijklmw	6.29 ± 0.35 abcdefghijklmnop
			Leaf, Top	8.69 ± 0.74 defghijklmnoqxy	22.57 ± 1.49 opqrstuvwxyz	68.74 ± 1.31 cdefghijklm	39.56 ± 1.11 iklmnopqrstuvwxyz	29.18 ± 0.84 abcdef	34.85 ± 0.93 efghijklmnopqrstw	4.71 ± 0.35 klmnopqr
			Stem, Bottom	2.40 ± 0.74 uvw	45.10 ± 1.49 bcd	52.50 ± 1.31 vwxy	35.81 ± 1.11 tuvwyza1b1c1d1e1f1	16.70 ± 0.84 qrs	29.26 ± 0.93 uvxyza1b1c1	6.55 ± 0.35 abcdefghijklmn
			Stem, Top	7.89 ± 0.74 efghijklmnoprxy	28.84 ± 1.49 ghijklmnopq	63.26 ± 1.31 jlmnopqrs	38.72 ± 1.11 mnopqrstuvwxyza1b1c1	24.55 ± 0.84 efghijklmno	33.91 ± 0.93 iklmnopqrstuv	4.82 ± 0.35 ijklmnopqr
		MOL 6136	Leaf, Bottom	12.98 ± 0.74 abcdq	16.29 ± 1.49 tuvwxy	70.73 ± 1.31 bcdefghij	44.46 ± 1.11 abcdefghijklmnpq	26.27 ± 0.84 defghijklmn	38.19 ± 0.93 abcdefghijklmw	6.26 ± 0.35 abcdefghijklmnop
			Leaf, Top	11.75 ± 0.74 abcdeq	20.54 ± 1.49 qrstuvwxyz	67.71 ± 1.31 defghijklmo	39.31 ± 1.11 iklmnopqrstuvwxyza1	28.40 ± 0.84 abcdefghijk	34.18 ± 0.93 efghijklmnopqrstuw	5.13 ± 0.35 hijklmnopqr
			Stem, Bottom	3.99 ± 0.74 prstuvwy	41.12 ± 1.49 cde	54.89 ± 1.31 tuvwx	38.38 ± 1.11 orstuvwxyza1b1c1	16.51 ± 0.84 qrs	31.51 ± 0.93 qrstuvxyzb1c1	6.87 ± 0.35 abcdefghi
			Stem, Top	9.35 ± 0.74 abcdefghijklmnqx	24.49 ± 1.49 klmnopqrstuvwxyz	66.16 ± 1.31 defghijklmnopq	40.05 ± 1.11 hijklmnopqrstuvwxyz	26.11 ± 0.84 defghijklmno	34.83 ± 0.93 efghijklmnopqrstw	5.23 ± 0.35 ghijklmnopqr

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the p ≤ 0.05 level

Table B.1. (Continued) Least square means of composition of different plant parts of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2015	305	77-9271	Leaf, Bottom	5.00 ± 0.74 noprstuvwy	15.60 ± 1.49 x	79.40 ± 1.31 a	50.16 ± 1.11 a	29.24 ± 0.84 abcdefg	42.31 ± 0.93 ac	7.85 ± 0.35 ab
			Leaf, Top	9.97 ± 0.74 abcdefghijklmq	17.72 ± 1.49 stuvwxy	72.31 ± 1.31 abcdefg	41.37 ± 1.11 defghijklmnopqrstuwxz	30.94 ± 0.84 abcd	35.35 ± 0.93 efghijklmnopqrsw	6.03 ± 0.35 bcdefghijklmnopqr
			Stem, Bottom	4.89 ± 0.74 noprstuvwy	45.24 ± 1.49 bcd	49.87 ± 1.31 wxyz	34.9 ± 1.11 vya1b1c1d1e1f1	14.97 ± 0.84 r	27.97 ± 0.93 xza1c1d1	6.93 ± 0.35 abcdefgh
			Stem, Top	11.22 ± 0.74 abcdefq	24.63 ± 1.49 klmnopqrstuvwyz	64.16 ± 1.31 hijklmnopqr	38.82 ± 1.11 kmnopqrstuvwxyza1c1	25.35 ± 0.84 efghijklmno	34.00 ± 0.93 efghijklmnopqrstuw	4.81 ± 0.35 ijklmnopqr
		MOL 6136	Leaf, Bottom	5.75 ± 0.74 klmnoprstuvwxy	16.04 ± 1.49 vwxy	78.21 ± 1.31 ab	50.00 ± 1.11 ab	28.21 ± 0.84 bcdefghijk	42.43 ± 0.93 a	7.57 ± 0.35 abcde
			Leaf, Top	12.97 ± 0.74 abcdq	16.47 ± 1.49 uvwxy	70.56 ± 1.31 cdefghijk	38.67 ± 1.11 mnopqrstuvwxyza1c1	31.89 ± 0.84 abc	33.98 ± 0.93 gjklmnopqrstu	4.70 ± 0.35 lmnopqr
			Stem, Bottom	4.89 ± 0.74 noprstuvwy	45.62 ± 1.49 bcd	49.48 ± 1.31 wxyz	35.08 ± 1.11 uvya1b1c1d1e1f1	14.40 ± 0.84 r	28.36 ± 0.93 vxyza1b1c1	6.73 ± 0.35 abcdefghijkl
			Stem, Top	9.82 ± 0.74 abcdefghijklq	24.40 ± 1.49 klmnopqrstuvwyz	65.78 ± 1.31 fghijklmnopq	39.22 ± 1.11 kmnopqrstuvwxyza1	26.56 ± 0.84 defghijklmn	34.23 ± 0.93 efghijklmnopqrstuw	4.99 ± 0.35 hijklmnopqr

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the p ≤ 0.05 level

Table B.1. (Continued) Least square means of composition of different plant parts of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2015	915	77-9271	Leaf, Bottom	8.14 ± 0.74 efghijklmnoprxy	20.63 ± 1.49 qrstuvwxyz	71.24 ± 1.31 bcdefghij	44.85 ± 1.11 abcdefghijklmop	26.39 ± 0.84 defghijklmn	38.09 ± 0.93 abcdefghijklmnow	6.76 ± 0.35 abcdefghijkl
			Leaf, Top	7.92 ± 0.74 efghijklmnoprxy	23.63 ± 1.49 klmnopqrstuvwxyz	68.45 ± 1.31 defghijklm	41.63 ± 1.11 eghijklmnopqrstuvxz	26.83 ± 0.84 cdefghijklmn	36.40 ± 0.93 efghijklmnopqw	5.22 ± 0.35 ghijklmnopqr
			Stem, Bottom	1.47 ± 0.74 vw	50.66 ± 1.49 ab	47.87 ± 1.31 xyz	33.38 ± 1.11 ya1b1c1d1e1f1	14.49 ± 0.84 r	27.88 ± 0.93 yza1c1	5.49 ± 0.35 efghijklmnopqr
			Stem, Top	7.59 ± 0.74 efghijklmnoprsxy	31.93 ± 1.49 fghijkln	60.48 ± 1.31 opqrstu	39.35 ± 1.11 iklmnopqrstuvwxyz	21.12 ± 0.84 opq	35.34 ± 0.93 efghijklmnopqrstw	4.02 ± 0.35 r
		MOL 6136	Leaf, Bottom	8.68 ± 0.74 defghijklmnox	19.28 ± 1.49 rstuvwxy	72.03 ± 1.31 abcdefgh	45.57 ± 1.11 abcdefghijk	26.46 ± 0.84 defghijklmn	39.18 ± 0.93 abcdefgi	6.39 ± 0.35 abcdefghijklmno
			Leaf, Top	9.02 ± 0.74 cdefghijklmnqx	24.14 ± 1.49 klmnopqrstuvwxyz	66.83 ± 1.31 defghijklmnop	38.46 ± 1.11 nqrstuvwxyza1b1c1	28.38 ± 0.84 abcdefghijk	33.53 ± 0.93 jklmnopqrstuvxb1	4.93 ± 0.35 hijklmnopqr
			Stem, Bottom	2.17 ± 0.74 uvw	48.70 ± 1.49 abc	49.13 ± 1.31 wxyz	32.57 ± 1.11 a1c1d1e1f1g1	16.57 ± 0.84 qrs	27.03 ± 0.93 za1d1	5.53 ± 0.35 defghijklmnopqr
			Stem, Top	8.72 ± 0.74 defghijklmnox	29.92 ± 1.49 fghijklmnop	61.36 ± 1.31 mnopqrstu	38.97 ± 1.11 mnopqrstuvwxyzb1	22.39 ± 0.84 mno	34.82 ± 0.93 efghijklmnopqrstuw	4.15 ± 0.35 qr

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the p ≤ 0.05 level

Table B.2. Least square means of composition of leaves and stems of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2013	30	77-9271	Leaf	8.43 ± 0.56 bcdefghi	21.37 ± 1.24 h	70.21 ± 1.10 abcde	43.53 ± 1.14 abc	26.68 ± 0.73 c	37.59 ± 0.90 ab	5.94 ± 0.32 bcdefghi
			Stem	2.53 ± 0.70 klm	46.42 ± 1.53 abcd	50.95 ± 1.35 ijkl	38.04 ± 1.38 bcdefghijk	13.05 ± 0.91 e	30.77 ± 1.09 defghijkl	7.20 ± 0.38 abci
		MOL 6136	Leaf	10.77 ± 0.56 abcd	19.80 ± 1.24 h	69.44 ± 1.10 bcde	43.07 ± 1.14 abc	26.37 ± 0.73 c	37.51 ± 0.90 ab	5.56 ± 0.32 bcdefghi
			Stem	5.68 ± 0.70 fghijkl	37.55 ± 1.53 efg	56.53 ± 1.35 ghi	40.38 ± 1.38 abcdefgh	16.24 ± 0.91 de	32.95 ± 1.09 bcdefghij	7.34 ± 0.38 ab
	305	77-9271	Leaf	4.11 ± 0.56 jklm	22.68 ± 1.24 h	73.21 ± 1.10 abcd	41.62 ± 1.14 abcdf	31.59 ± 0.73 ab	35.83 ± 0.90 abcd	5.79 ± 0.32 bcdefghi
			Stem	1.40 ± 0.56 m	48.89 ± 1.24 abc	49.71 ± 1.10 ijkl	33.01 ± 1.14 ijkl	16.70 ± 0.73 de	26.65 ± 0.90 klm	6.37 ± 0.32 abcdefi
		MOL 6136	Leaf	5.64 ± 0.56 gijk	22.78 ± 1.24 h	71.58 ± 1.10 abcde	39.74 ± 1.14 bcdefgh	31.83 ± 0.73 a	34.64 ± 0.90 abcdef	5.10 ± 0.32 efgh
			Stem	2.94 ± 0.70 klm	50.30 ± 1.53 abc	46.82 ± 1.36 klm	30.14 ± 1.38 kl	16.75 ± 0.91 de	25.40 ± 1.09 lm	4.76 ± 0.38 fgh

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.2. (Continued) Least square means of composition of leaves and stems of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2013	915	77-9271	Leaf	10.07 ± 0.56 abcde	22.25 ± 1.24 h	67.69 ± 1.10 de	40.36 ± 1.14 abcdef	27.33 ± 0.73 bc	35.24 ± 0.90 abcde	5.11 ± 0.32 defgh
			Stem	3.56 ± 0.56 klm	45.77 ± 1.24 bcd	50.67 ± 1.10 ijkl	33.61 ± 1.14 ghijkl	17.06 ± 0.73 de	28.35 ± 0.90 hjklm	5.26 ± 0.32 cdefghi
		MOL 6136	Leaf	11.06 ± 0.70 abcd	23.71 ± 1.53 h	65.50 ± 1.36 ef	38.96 ± 1.38 bcdefghi	26.62 ± 0.91 c	34.01 ± 1.09 abcdefgi	5.01 ± 0.38 defghi
			Stem	5.19 ± 0.70 hijkl	47.34 ± 1.53 abcd	47.45 ± 1.36 klm	31.69 ± 1.38 ijkl	15.77 ± 0.91 de	27.39 ± 1.09 jklm	4.24 ± 0.38 gh
2014	30	77-9271	Leaf	8.79 ± 0.56 bcdefgh	19.97 ± 1.24 h	71.24 ± 1.10 abcde	44.08 ± 1.14 ab	27.16 ± 0.73 c	37.45 ± 0.90 ab	6.63 ± 0.32 abcdefi
			Stem	2.12 ± 0.56 lm	48.30 ± 1.24 abc	49.58 ± 1.10 jkl	35.04 ± 1.14 fghijk	14.54 ± 0.73 de	28.58 ± 0.90 ghijklm	6.46 ± 0.32 abcdefi
		MOL 6136	Leaf	11.34 ± 0.56 abc	21.19 ± 1.24 h	67.47 ± 1.10 cde	41.62 ± 1.14 abcde	25.85 ± 0.73 c	35.38 ± 0.90 abcde	6.23 ± 0.32 abcdefgi
			Stem	4.55 ± 0.56 jklm	34.57 ± 1.24 g	60.89 ± 1.10 fg	43.09 ± 1.14 abc	17.8 ± 0.73 de	35.23 ± 0.90 abcde	7.86 ± 0.32 a

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.2. (Continued) Least square means of composition of leaves and stems of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2014	305	77-9271	Leaf	9.34 ± 0.56 abcdef	19.00 ± 1.24 h	71.67 ± 1.10 abcde	42.70 ± 1.14 abc	28.97 ± 0.73 abc	36.02 ± 0.90 abcd	6.69 ± 0.32 abcdei
			Stem	8.05 ± 0.56 defghi	36.84 ± 1.24 fg	55.12 ± 1.10 ghij	37.24 ± 1.14 cdefghij	17.88 ± 0.73 d	30.67 ± 0.90 efghijkl	6.58 ± 0.32 abcdefi
		MOL 6136	Leaf	11.49 ± 0.56 ab	16.57 ± 1.24 h	71.94 ± 1.10 abcde	42.96 ± 1.14 abc	28.97 ± 0.73 abc	36.68 ± 0.90 abc	6.28 ± 0.32 abcdefi
			Stem	8.14 ± 0.56 cdefghi	33.38 ± 1.24 g	58.48 ± 1.10 fgh	40.19 ± 1.14 bcdefg	18.28 ± 0.73 d	33.55 ± 0.90 bcdefgh	6.65 ± 0.32 abcdei
	915	77-9271	Leaf	7.16 ± 0.56 efghij	19.44 ± 1.24 h	73.40 ± 1.10 abc	44.97 ± 1.14 ab	28.43 ± 0.73 abc	38.99 ± 0.90 a	6.06 ± 0.32 abcdefgi
			Stem	2.07 ± 0.56 lm	51.67 ± 1.24 ab	46.27 ± 1.10 lm	31.27 ± 1.14 jkl	14.99 ± 0.73 de	26.31 ± 0.90 lm	4.96 ± 0.32 efgh
		MOL 6136	Leaf	8.82 ± 0.56 bcdefg	20.57 ± 1.24 h	70.61 ± 1.10 abcde	43.45 ± 1.14 abc	27.15 ± 0.73 c	38.18 ± 0.90 ab	5.27 ± 0.32 cdefghi
			Stem	2.90 ± 0.56 klm	54.40 ± 1.24 a	42.71 ± 1.10 m	27.99 ± 1.14 l	14.72 ± 0.73 de	23.72 ± 0.90 m	4.26 ± 0.32 h

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the p ≤ 0.05 level

Table B.2. (Continued) Least square means of composition of leaves and stems of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2015	30	77-9271	Leaf	9.50 ± 0.56 abcdef	20.84 ± 1.24 h	69.66 ± 1.10 bcde	42.08 ± 1.14 abc	27.58 ± 0.73 abc	36.65 ± 0.90 abc	5.43 ± 0.32 cdefghi
			Stem	2.81 ± 0.56 klm	43.86 ± 1.24 cdef	53.34 ± 1.10 hijk	36.04 ± 1.14 defghijk	17.29 ± 0.73 de	29.61 ± 0.90 fghijkl	6.43 ± 0.32 abcdefi
		MOL 6136	Leaf	12.44 ± 0.56 a	18.24 ± 1.24 h	69.31 ± 1.10 bcde	42.05 ± 1.14 abcd	27.26 ± 0.73 c	36.32 ± 0.90 abc	5.73 ± 0.32 bcdefghi
			Stem	4.42 ± 0.56 jklm	39.74 ± 1.24 defg	55.84 ± 1.10 ghi	38.54 ± 1.14 bcdefghi	17.29 ± 0.73 de	31.8 ± 0.90 cdefghijk	6.75 ± 0.32 abcdefi
	305	77-9271	Leaf	7.03 ± 0.56 efghij	16.53 ± 1.24 h	76.44 ± 1.10 a	46.55 ± 1.14 a	29.89 ± 0.73 abc	39.48 ± 0.90 a	7.08 ± 0.32 abc
			Stem	5.22 ± 0.56 ijkl	44.19 ± 1.24 cde	50.59 ± 1.10 ijkl	35.10 ± 1.14 eghijk	15.49 ± 0.73 de	28.28 ± 0.90 ijklm	6.82 ± 0.32 abcdi
		MOL 6136	Leaf	8.80 ± 0.56 bcdefh	16.33 ± 1.24 h	74.87 ± 1.10 ab	45.14 ± 1.14 ab	29.73 ± 0.73 abc	38.80 ± 0.90 a	6.34 ± 0.32 abcdefi
			Stem	5.20 ± 0.56 ijkl	44.41 ± 1.24 cde	50.39 ± 1.10 ijkl	35.31 ± 1.14 eghijk	15.09 ± 0.73 de	28.68 ± 0.90 ijklm	6.63 ± 0.32 abcdei

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the p ≤ 0.05 level

Table B.2. (Continued) Least square means of composition of leaves and stems of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2015	915	77-9271	Leaf	7.99 ± 0.56 defghi	22.39 ± 1.24 h	69.62 ± 1.10 bcde	42.95 ± 1.14 abc	26.67 ± 0.73 c	37.09 ± 0.90 ab	5.86 ± 0.32 bcdefghi
			Stem	2.15 ± 0.56 lm	48.56 ± 1.24 abc	49.29 ± 1.10 jkl	34.10 ± 1.14 ghijk	15.19 ± 0.73 de	28.78 ± 0.90 ghijkl	5.32 ± 0.32 bcdefghi
		MOL 6136	Leaf	8.93 ± 0.56 bcdef	21.89 ± 1.24 h	69.18 ± 1.10 bcde	41.69 ± 1.14 abcde	27.50 ± 0.73 bc	36.10 ± 0.90 abcd	5.59 ± 0.32 bcdefghi
			Stem	2.82 ± 0.56 klm	46.83 ± 1.24 bcd	50.35 ± 1.10 ijkl	33.21 ± 1.14 hijkl	17.14 ± 0.73 de	27.81 ± 0.90 jklm	5.40 ± 0.32 bcdefghi

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the p ≤ 0.05 level

Table B.3. Least square means of composition of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2013	30	77-9271	3.03 ± 0.68^{cdef}	43.01 ± 1.69^{abcd}	53.92 ± 1.49^{cde}	38.71 ± 1.47^{abcd}	15.19 ± 0.83^{c}	31.68 ± 1.17^{abcde}	6.99 ± 0.41^{abc}
		MOL 6136	6.35 ± 0.68^{abcde}	34.67 ± 1.69^{defgh}	58.36 ± 1.49^{abcd}	41.14 ± 1.47^{abc}	17.55 ± 0.83^{abc}	33.92 ± 1.17^{abc}	7.19 ± 0.41^{ab}
	305	77-9271	1.86 ± 0.58^{f}	44.49 ± 1.33^{ab}	53.65 ± 1.13^{cde}	34.45 ± 1.19^{cd}	19.2 ± 0.66^{abc}	28.19 ± 0.94^{cde}	6.27 ± 0.34 ^{abcde}
		MOL 6136	3.88 ± 0.63^{bcdef}	44.43 ± 1.71^{abc}	51.61 ± 1.72^{de}	31.84 ± 1.48^{d}	20.31 ± 0.84^{ab}	27.15 ± 1.18^{de}	4.77 ± 0.41^{ce}
	915	77-9271	5.12 ± 0.58^{bcde}	40.10 ± 1.33^{bcde}	54.78 ± 1.13^{cd}	35.21 ± 1.19^{bcd}	19.57 ± 0.66^{ab}	29.99 ± 0.94^{bcde}	5.22 ± 0.34^{bcde}
		MOL 6136	7.72 ± 0.76^{ab}	45.03 ± 2.51^{abcd}	47.20 ± 2.82^{cde}	31.74 ± 2.12^{bcd}	17.38 ± 1.21^{abc}	27.56 ± 1.70^{bcde}	4.20 ± 0.58^{de}
2014	30	77-9271	3.75 ± 0.58^{cdef}	41.39 ± 1.33^{abcd}	54.86 ± 1.13^{cde}	37.22 ± 1.19^{abcd}	17.64 ± 0.66^{bc}	30.72 ± 0.94^{abcde}	6.51 ± 0.34^{abcd}
		MOL 6136	6.86 ± 0.58^{abc}	30.00 ± 1.33^{gh}	63.14 ± 1.13^{a}	42.60 ± 1.19^{a}	20.54 ± 0.66^{ab}	35.28 ± 0.94^{a}	7.32 ± 0.34^{a}
	305	77-9271	8.38 ± 0.58^{a}	31.93 ± 1.33^{fgh}	59.68 ± 1.13^{abc}	38.75 ± 1.19^{abc}	20.93 ± 0.66^{ab}	32.14 ± 0.94^{abcd}	6.61 ± 0.34^{abd}
		MOL 6136	9.18 ± 0.58^{a}	28.27 ± 1.33^{h}	62.55 ± 1.13^{ab}	41.04 ± 1.19^{ab}	21.51 ± 0.66^{a}	34.50 ± 0.94^{ab}	6.54 ± 0.34^{abcd}
	915	77-9271	3.41 ± 0.58^{ef}	43.14 ± 1.33^{abc}	53.44 ± 1.13^{cde}	34.89 ± 1.19^{bcd}	18.55 ± 0.66^{abc}	29.63 ± 0.94^{bcde}	5.25 ± 0.34^{bcde}
		MOL 6136	4.14 ± 0.58^{bcdef}	47.24 ± 1.33^{a}	48.62 ± 1.13^{e}	31.31 ± 1.19^{d}	17.31 ± 0.66^{bc}	26.81 ± 0.94^{e}	4.49 ± 0.34^{e}

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.3. (Continued) Least square means of composition of the Energycane cultivars collected across the three elevations and harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2015	30	77-9271	4.25 ± 0.58^{bcdef}	38.94 ± 1.33^{bcdef}	56.81 ± 1.13^{bcd}	37.33 ± 1.19^{abcd}	19.48 ± 0.66^{ab}	31.10 ± 0.94^{abcde}	6.23 ± 0.34^{abcde}
		MOL 6136	6.63 ± 0.58^{abcd}	33.82 ± 1.33^{efgh}	59.55 ± 1.13^{abcd}	39.53 ± 1.19^{abc}	20.03 ± 0.66^{ab}	33.05 ± 0.94^{abcd}	6.47 ± 0.34^{abcd}
	305	77-9271	5.65 ± 0.58^{abcde}	37.47 ± 1.33^{bcdef}	56.88 ± 1.13^{abcd}	37.89 ± 1.19^{abcd}	18.98 ± 0.66^{abc}	31.01 ± 0.94^{abcde}	6.88 ± 0.34^{ab}
		MOL 6136	6.16 ± 0.58^{abcde}	36.94 ± 1.33^{cdefg}	56.90 ± 1.13^{abcd}	37.96 ± 1.19^{abcd}	18.95 ± 0.66^{abc}	31.40 ± 0.94^{abcde}	6.55 ± 0.34^{abcd}
	915	77-9271	3.46 ± 0.58^{def}	42.69 ± 1.33^{abcd}	53.84 ± 1.13^{cde}	36.08 ± 1.19^{abcd}	17.76 ± 0.66^{bc}	30.64 ± 0.94^{abcde}	5.44 ± 0.34^{bcde}
		MOL 6136	3.98 ± 0.58^{bcdef}	42.08 ± 1.33^{abcd}	53.95 ± 1.13^{cde}	34.83 ± 1.19^{bcd}	19.12 ± 0.66^{abc}	29.40 ± 0.94^{bcde}	5.42 ± 0.34^{bcde}

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.4. Least square means of composition of plant part of the Napier grass cultivars collected across the three elevations, two harvest years and two harvest seasons (Unit: % dry matter)

Harvest year	Harvest season	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2014	March	30	Green	Leaf	4.69 ± 1.25 klm	30.00 ± 1.60 abc	65.31 ± 1.21 lmnopqrs	39.24 ± 1.29 lmn	26.08 ± 0.94 abcdefgh	35.09 ± 0.90 klmnop	4.15 ± 0.51 ij
				Stem	14.85 ± 1.25 abcde	10.20 ± 1.60 p	74.95 ± 1.21 abcdef	52.26 ± 1.29 defg	22.69 ± 0.94 defghijklm	43.37 ± 0.90 cdef	8.89 ± 0.51 bcdef
			Purple	Leaf	4.77 ± 1.25 klm	31.26 ± 1.60 a	63.97 ± 1.21 rs	38.45 ± 1.29 mn	25.52 ± 0.94 abcdefghi	34.21 ± 0.90 mnop	4.24 ± 0.5 hij
				Stem	13.49 ± 1.25 abcdefgi	18.04 ± 1.60 gijklmnop	68.46 ± 1.21 fghijklmnopqrs	47.81 ± 1.29 fghij	20.65 ± 0.94 hijklmn	39.61 ± 0.90 defghijkl	8.20 ± 0.51 cdef
		305	Green	Leaf	7.24 ± 1.25 fghijklm	27.77 ± 1.60 abcd	64.99 ± 1.21 opqrs	37.04 ± 1.29 mn	27.95 ± 0.94 abcd	33.18 ± 0.90 op	3.86 ± 0.51 j
				Stem	6.76 ± 1.49 fghijklm	22.27 ± 1.94 abcdefghijklmn	70.14 ± 1.48 cdefghijklmnopqrs	48.51 ± 1.60 efghijk	21.49 ± 1.16 efghijklmn	41.00 ± 1.11 cdefghijk	7.50 ± 0.62 efgh
			Purple	Leaf	3.43 ± 1.25 lm	30.54 ± 1.60 ab	66.03 ± 1.21 klmnopqrs	37.67 ± 1.29 mn	28.37 ± 0.94 abc	33.73 ± 0.90 nop	3.93 ± 0.51 j
				Stem	6.03 ± 1.25 jklm	26.94 ± 1.60 abcdefgh	67.03 ± 1.21 hijklmnopqrs	46.36 ± 1.29 ghijkl	20.66 ± 0.94 hijklmn	39.41 ± 0.90 efghijklm	6.95 ± 0.51 fghi

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the p ≤ 0.05 level

Table B.4. (Continued) Least square means of composition of plant part of the Napier grass cultivars collected across the three elevations, two harvest years and two harvest seasons (Unit: % dry matter)

Harvest year	Harvest season	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2014	March	915	Green	Leaf	15.17 ± 1.25 abcd	22.12 ± 1.60 abcdefghijklmn	62.71 ± 1.21 s	38.21 ± 1.29 mn	24.50 ± 0.94 abcdefghijk	33.96 ± 0.90 nop	4.24 ± 0.51 hij
				Stem	5.41 ± 1.25 jklm	23.46 ± 1.60 abcdefghijk	71.14 ± 1.21 cdefghijklmnop	51.96 ± 1.29 defg	19.17 ± 0.94 lmn	43.55 ± 0.90 cdef	8.41 ± 0.51 cdef
			Purple	Leaf	13.91 ± 1.25 abcdefgh	23.78 ± 1.60 abcdefghij	62.31 ± 1.21 s	38.26 ± 1.29 mn	24.04 ± 0.94 bcdefghijkl	33.33 ± 0.90 op	4.94 ± 0.51 hij
				Stem	5.32 ± 1.25 jklm	21.04 ± 1.60 cdefghijklmn	73.64 ± 1.21 abcdefghi	54.28 ± 1.29 bcdef	19.36 ± 0.94 klmn	44.71 ± 0.90 abcd	9.57 ± 0.51 bcdef
	Sept.	30	Green	Leaf	4.29 ± 1.25 lm	27.50 ± 1.60 abcdefg	68.21 ± 1.21 ghijklmnopqrs	41.67 ± 1.29 ijklmn	26.54 ± 0.94 abcdef	37.12 ± 0.90 hijklmno	4.55 ± 0.51 hij
				Stem	9.21 ± 1.25 cefghijklm	14.08 ± 1.60 klmnop	76.71 ± 1.21 abcde	56.33 ± 1.29 abcde	20.39 ± 0.94 ijklmn	46.10 ± 0.90 abc	10.23 ± 0.51 abcde
			Purple	Leaf	6.35 ± 1.25 ijklm	26.96 ± 1.60 abcdefh	66.69 ± 1.21 hijklmnopqrs	40.28 ± 1.29 klmn	26.41 ± 0.94 abcdef	35.07 ± 0.90 klmnop	5.21 ± 0.51 ghij
				Stem	8.54 ± 1.25 bdefghijklm	20.97 ± 1.60 cdefghijklmn	70.49 ± 1.21 defghijklmnopq	53.83 ± 1.29 bcdefg	16.66 ± 0.94 n	43.51 ± 0.90 cdef	10.32 ± 0.51 abcde

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.4. (Continued) Least square means of composition of plant part of the Napier grass cultivars collected across the three elevations, two harvest years and two harvest seasons (Unit: % dry matter)

Harvest year	Harvest season	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2014	Sept.	305	Green	Leaf	6.87 ± 1.25 ghijklm	26.48 ± 1.60 abcdefghi	66.65 ± 1.21 ijklmnopqrs	37.20 ± 1.29 mn	29.44 ± 0.94 ab	33.28 ± 0.90 nop	3.92 ± 0.51 ij
				Stem	9.03 ± 1.25 abcdefghijklm	18.43 ± 1.60 efhijklmnop	72.53 ± 1.21 abcdefghijk	51.99 ± 1.29 defg	20.55 ± 0.94 ijklmn	42.14 ± 0.9 cdefgh	9.85 ± 0.51 abcdef
			Purple	Leaf	5.10 ± 1.25 klm	29.88 ± 1.60 abc	65.02 ± 1.21 opqrs	37.54 ± 1.29 mn	27.48 ± 0.94 abcde	33.23 ± 0.90 nop	4.31 ± 0.51 hij
				Stem	14.60 ± 1.25 abcdf	13.70 ± 1.60 lmnop	71.70 ± 1.21 cdefghijklmn	51.93 ± 1.29 defg	19.78 ± 0.94 jklmn	41.81 ± 0.90 cdefgh	10.12 ± 0.51 abcde
		915	Green	Leaf	12.76 ± 1.25 abcdefghij	19.94 ± 1.60 defghijklmno	67.31 ± 1.21 hijklmnopqrs	42.88 ± 1.29 hijklm	24.43 ± 0.94 abcdefghijkl	38.48 ± 0.90 fghijklmn	4.40 ± 0.51 hij
				Stem	4.22 ± 1.25 klm	16.91 ± 1.60 jklmnop	78.88 ± 1.21 ab	59.27 ± 1.29 abcd	19.61 ± 0.94 klmn	49.09 ± 0.90 ab	10.17 ± 0.51 abcde
			Purple	Leaf	14.08 ± 1.25 abcdefgh	22.22 ± 1.60 abcdefghijklm	63.71 ± 1.21 qrs	39.26 ± 1.29 lmn	24.45 ± 0.94 abcdefghijkl	34.93 ± 0.90 lmnop	4.33 ± 0.51 hij
				Stem	4.58 ± 1.25 klm	17.75 ± 1.60 hijklmnop	77.67 ± 1.21 abc	59.89 ± 1.29 abc	17.77 ± 0.94 mn	48.87 ± 0.90 ab	11.02 ± 0.51 abc

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the p ≤ 0.05 level

Table B.4. (Continued) Least square means of composition of plant part of the Napier grass cultivars collected across the three elevations, two harvest years and two harvest seasons (Unit: % dry matter)

Harvest year	Harvest season	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2015	March	30	Green	Leaf	7.08 ± 1.25 ghijklm	27.66 ± 1.60 abcdeg	65.26 ± 1.21 mnpqrs	38.04 ± 1.29 mn	27.22 ± 0.94 abcde	34.38 ± 0.90 mnop	3.66 ± 0.51 j
				Stem	15.32 ± 1.25 abd	12.94 ± 1.60 mnop	71.74 ± 1.21 cdefghijklo	52.29 ± 1.29 defg	19.44 ± 0.94 klmn	42.18 ± 0.90 cdefgh	10.12 ± 0.51 abcde
			Purple	Leaf	7.83 ± 1.25 defghijklm	27.19 ± 1.60 abcdef	64.99 ± 1.21 npqrs	38.44 ± 1.29 mn	26.54 ± 0.94 abcdef	34.59 ± 0.90 lmnop	3.85 ± 0.51 j
				Stem	15.96 ± 1.25 ac	13.71 ± 1.60 lmnop	70.34 ± 1.21 efghijklmnopqr	48.89 ± 1.29 efghi	21.44 ± 0.94 fghijklmn	40.43 ± 0.90 defghij	8.47 ± 0.51 bcdef
		305	Green	Leaf	5.88 ± 1.25 jklm	27.33 ± 1.60 abcdg	66.79 ± 1.21 ijklmnopqrs	40.56 ± 1.29 jklmn	26.24 ± 0.94 abcdefg	35.54 ± 0.90 jklmnop	5.02 ± 0.51 hij
				Stem	8.43 ± 1.25 bdefghijklm	18.21 ± 1.60 fhijklmnop	73.36 ± 1.21 abcdefgh	52.92 ± 1.29 cdefg	20.44 ± 0.94 ijklmn	41.95 ± 0.90 cdefgh	10.97 ± 0.51 abcd
			Purple	Leaf	3.68 ± 1.25 lm	31.22 ± 1.60 a	65.10 ± 1.21 opqrs	38.51 ± 1.29 mn	26.6 ± 0.94 abcdef	34.07 ± 0.90 nop	4.43 ± 0.51 hij
				Stem	7.71 ± 1.25 eijklm	19.56 ± 1.60 defghijklmnop	72.74 ± 1.21 abcdefghij	51.87 ± 1.29 defg	20.87 ± 0.94 ghijklmn	41.57 ± 0.90 cdefgh	10.29 ± 0.51 abcde

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the p ≤ 0.05 level

Table B.4. (Continued) Least square means of composition of plant part of the Napier grass cultivars collected across the three elevations, two harvest years and two harvest seasons (Unit: % dry matter)

Harvest year	Harvest season	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2015	March	915	Green	Leaf	15.30 ± 1.25 abc	22.34 ± 1.60 abcdefghijkl	62.36 ± 1.21 s	35.33 ± 1.29 n	27.02 ± 0.94 abcde	31.31 ± 0.90 p	4.03 ± 0.51 ij
				Stem	5.91 ± 1.25 jklm	22.76 ± 1.60 abcdefghijkl	71.33 ± 1.21 cdefghijklmno	50.02 ± 1.29 efgh	21.31 ± 0.94 fghijklmn	42.02 ± 0.90 cdefgh	8.00 ± 0.51 cdefg
			Purple	Leaf	11.70 ± 1.25 abcdefghijk	25.29 ± 1.60 abcdefghij	63.01 ± 1.21 s	35.70 ± 1.29 mn	27.31 ± 0.94 abcde	31.30 ± 0.90 p	4.41 ± 0.51 hij
				Stem	4.15 ± 1.25 lm	24.12 ± 1.60 abcdefghij	71.73 ± 1.21 cdefghijklmno	49.05 ± 1.29 efghi	22.68 ± 0.94 defghijklm	41.01 ± 0.90 cdefghi	8.03 ± 0.51 defg
	Sept.	30	Green	Leaf	3.56 ± 1.25 lm	30.29 ± 1.60 abc	66.15 ± 1.21 jklmnopqrs	41.86 ± 1.29 ijklmn	24.29 ± 0.94 abcdefghijkl	37.89 ± 0.90 ghijklmno	3.97 ± 0.51 j
				Stem	10.26 ± 1.25 abcdefghijkl	12.79 ± 1.60 nop	76.96 ± 1.21 abcd	54.74 ± 1.29 abcdef	22.22 ± 0.94 efghijklm	44.54 ± 0.90 abcde	10.20 ± 0.51 abcde
			Purple	Leaf	4.48 ± 1.25 klm	31.47 ± 1.60 a	64.05 ± 1.21 pqrs	40.21 ± 1.29 klmn	23.84 ± 0.94 cdefghijkl	35.77 ± 0.90 ijklmnop	4.44 ± 0.51 hij
				Stem	6.91 ± 1.25 hjklm	21.32 ± 1.60 bcdefghijklmn	71.78 ± 1.21 bcdefghijklmo	51.80 ± 1.29 defg	19.98 ± 0.94 jklmn	41.66 ± 0.90 cdefgh	10.13 ± 0.51 abcde

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.4. (Continued) Least square means of composition of plant part of the Napier grass cultivars collected across the three elevations, two harvest years and two harvest seasons (Unit: % dry matter)

Harvest year	Harvest season	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2015	Sept.	305	Green	Leaf	7.31 ± 1.25 fghijklm	21.30 ± 1.60 bcdefghijklmn	71.39 ± 1.21 cdefghijklmno	41.68 ± 1.29 ijklmn	29.71 ± 0.94 a	37.09 ± 0.90 hijklmno	4.59 ± 0.51 hij
				Stem	10.20 ± 1.25 abcdefghijkl	13.41 ± 1.60 lmnop	76.38 ± 1.21 abcde	54.11 ± 1.29 bcdef	22.28 ± 0.94 efghijklm	43.15 ± 0.90 cdefg	10.96 ± 0.51 abcd
			Purple	Leaf	4.23 ± 1.25 lm	26.72 ± 1.60 abcdefghi	69.06 ± 1.21 fghijklmnopqrs	40.49 ± 1.29 jklmn	28.57 ± 0.94 abc	35.87 ± 0.90 ijklmnop	4.62 ± 0.51 hij
				Stem	13.91 ± 1.25 abcdfgh	11.39 ± 1.60 op	74.71 ± 1.21 abcdefg	54.05 ± 1.29 bcdef	20.66 ± 0.94 hijklmn	43.88 ± 0.90 bcde	10.16 ± 0.51 abcde
		915	Green	Leaf	12.52 ± 1.25 abcdefghij	21.68 ± 1.60 bcdefghijklmn	65.81 ± 1.21 jklmnopqrs	40.51 ± 1.29 jklmn	25.29 ± 0.94 abcdefghij	35.58 ± 0.90 jklmnop	4.94 ± 0.51 hij
				Stem	3.64 ± 1.25 lm	17.43 ± 1.60 ijklmnop	78.92 ± 1.21 a	60.63 ± 1.29 ab	18.29 ± 0.94 mn	49.41 ± 0.90 a	11.22 ± 0.51 ab
			Purple	Leaf	12.70 ± 1.25 abcdefghij	24.53 ± 1.60 abcdefghij	62.77 ± 1.21 s	38.61 ± 1.29 mn	24.16 ± 0.94 bcdefghijkl	33.97 ± 0.90 nop	4.65 ± 0.51 hij
				Stem	2.74 ± 1.25 m	17.69 ± 1.60 hijklmnop	79.58 ± 1.21 a	61.74 ± 1.29 a	17.83 ± 0.94 mn	49.24 ± 0.90 a	12.50 ± 0.51 a

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the p ≤ 0.05 level

Table B.5. Least square means of composition of the Napier grass cultivars collected across the three elevations, two harvest years and two harvest seasons (Unit: % dry matter)

Harvest year	Harvest season	Elevation (m)	Cultivar	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2014	March	30	Green	10.03 ± 0.88 abcd	19.63 ± 1.30 bcd	70.34 ± 1.04 abcdefghi	46.04 ± 1.22 defgh	24.30 ± 0.83 abc	39.42 ± 0.82 defg	6.62 ± 0.47 cdef
			Purple	9.84 ± 0.88 abcd	23.51 ± 1.30 abcd	66.65 ± 1.04 hi	43.99 ± 1.22 fgh	22.66 ± 0.83 abc	37.41 ± 0.82 fg	6.58 ± 0.47 cdef
		305	Green	8.24 ± 0.88 abcde	24.42 ± 1.30 abc	67.34 ± 1.04 fghi	42.58 ± 1.22 h	24.76 ± 0.83 ab	36.79 ± 0.82 g	5.79 ± 0.47 f
			Purple	4.92 ± 0.88 e	28.48 ± 1.30 a	66.60 ± 1.04 gi	42.80 ± 1.22 gh	23.80 ± 0.83 abc	37.08 ± 0.82 g	5.73 ± 0.47 ef
		915	Green	8.74 ± 0.88 abcde	23.06 ± 1.30 abcd	68.20 ± 1.04 efghi	47.35 ± 1.22 cdefgh	20.85 ± 0.83 abc	40.30 ± 0.82 cdefg	7.05 ± 0.47 bcdef
			Purple	7.98 ± 0.88 abcde	21.88 ± 1.30 abcd	70.14 ± 1.04 abcdefghi	49.33 ± 1.22 abcdefgh	20.81 ± 0.83 abc	41.19 ± 0.82 abcdefg	8.14 ± 0.47 abcdef
	Sept.	30	Green	7.37 ± 0.88 cde	19.06 ± 1.30 bcd	73.56 ± 1.04 abcde	50.92 ± 1.22 abcde	22.64 ± 0.83 abc	42.79 ± 0.82 abcde	8.14 ± 0.47 abcdef
			Purple	7.79 ± 0.88 bcde	22.95 ± 1.30 abcd	69.26 ± 1.04 abcdefghi	49.44 ± 1.22 abcdefg	19.82 ± 0.83 c	40.78 ± 0.82 bcdefg	8.66 ± 0.47 abcd

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.5. (Continued) Least square means of composition of the Napier grass cultivars collected across the three elevations, two harvest years and two harvest seasons (Unit: % dry matter)

Harvest year	Harvest season	Elevation (m)	Cultivar	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2014	Sept.	305	Green	8.27 ± 0.88 abcde	21.44 ± 1.30 abcd	70.29 ± 1.04 abcdefghi	46.30 ± 1.22 defgh	23.99 ± 0.83 abc	38.70 ± 0.82 efg	7.59 ± 0.47 abcdef
			Purple	10.92 ± 0.88 abc	20.01 ± 1.30 bcd	69.08 ± 1.04 befghi	46.30 ± 1.22 defgh	22.77 ± 0.83 abc	38.46 ± 0.82 efg	7.84 ± 0.47 abcdef
		915	Green	7.14 ± 0.88 cde	17.96 ± 1.30 cd	74.89 ± 1.04 a	53.64 ± 1.22 abc	21.26 ± 0.83 abc	45.46 ± 0.82 a	8.18 ± 0.47 abcdef
			Purple	8.06 ± 0.88 abcde	19.39 ± 1.30 bcd	72.55 ± 1.04 abcdef	52.35 ± 1.22 abcd	20.20 ± 0.83 bc	43.78 ± 0.82 abcd	8.57 ± 0.47 abcd
2015	March	30	Green	12.39 ± 0.88 ab	18.07 ± 1.30 bcd	69.53 ± 1.04 abcdefghi	47.36 ± 1.22 bcdefgh	22.17 ± 0.83 abc	39.49 ± 0.82 defg	7.88 ± 0.47 abcdef
			Purple	12.65 ± 0.88 a	19.19 ± 1.30 cd	68.15 ± 1.04 fghi	44.65 ± 1.22 efgh	23.51 ± 0.83 abc	38.06 ± 0.82 fg	6.59 ± 0.47 cdef
		305	Green	7.88 ± 0.88 abcde	20.30 ± 1.3bcd	71.82 ± 1.04 abcdefh	50.05 ± 1.22 abcdef	21.77 ± 0.83 abc	40.46 ± 0.82 bcdefg	9.60 ± 0.47 ab
			Purple	6.66 ± 0.88 cde	22.41 ± 1.30 bcd	70.92 ± 1.04 abcdefghi	48.73 ± 1.22 abcdefgh	22.20 ± 0.83 abc	39.83 ± 0.82 cdefg	8.90 ± 0.47 abcd

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.5. (Continued) Least square means of composition of the Napier grass cultivars collected across the three elevations, two harvest years and two harvest seasons (Unit: % dry matter)

Harvest year	Harvest season	Elevation (m)	Cultivar	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2015	March	915	Green	8.97 ± 0.88 abcde	22.67 ± 1.30 abcd	68.37 ± 1.04 efghi	45.25 ± 1.22 efgh	23.11 ± 0.83 abc	38.54 ± 0.82 efg	6.71 ± 0.47 def
			Purple	6.79 ± 0.88 cde	24.53 ± 1.30 abc	68.68 ± 1.04 defghi	44.39 ± 1.22 efgh	24.29 ± 0.83 abc	37.61 ± 0.82 fg	6.77 ± 0.47 cdef
	Sept.	30	Green	7.03 ± 0.88 cde	20.61 ± 1.30 bcd	72.36 ± 1.04 abcdefg	49.12 ± 1.22 abcdefgh	23.24 ± 0.83 abc	41.64 ± 0.82 abcdef	7.48 ± 0.47 abcdef
			Purple	5.91 ± 0.88 de	25.32 ± 1.30 ab	68.77 ± 1.04 cdefghi	47.27 ± 1.22 cdefgh	21.49 ± 0.83 abc	39.37 ± 0.82 defg	7.91 ± 0.47 abcdef
		305	Green	8.99 ± 0.88 abcde	16.74 ± 1.30 d	74.28 ± 1.04 acd	48.87 ± 1.22 abcdefgh	25.41 ± 0.83 a	40.59 ± 0.82 bcdefg	8.27 ± 0.47 abcde
			Purple	10.02 ± 0.88 abcd	17.53 ± 1.30 cd	72.45 ± 1.04 abcdef	48.57 ± 1.22 abcdefgh	23.87 ± 0.83 abc	40.65 ± 0.82 bcdefg	7.92 ± 0.47 abcdef
		915	Green	6.55 ± 0.88 cde	18.82 ± 1.30 bcd	74.63 ± 1.04 ab	54.06 ± 1.22 ab	20.57 ± 0.83 bc	44.89 ± 0.82 ab	9.17 ± 0.47 abc
			Purple	5.92 ± 0.88 de	19.87 ± 1.30 bcd	74.21 ± 1.04 abc	54.35 ± 1.22 a	19.86 ± 0.83 c	44.37 ± 0.82 abc	9.98 ± 0.47 a

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.6. Least square means of composition of the Napier grass cultivars collected across the three elevations and two harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Cultivar	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2014	30	Green	7.70 ± 0.75[a]	19.14 ± 1.09[a]	73.16 ± 0.78[a]	50.30 ± 0.75[abc]	22.86 ± 0.58[ab]	42.36 ± 0.48[ab]	7.95 ± 0.34[ab]
		Purple	8.24 ± 0.75[a]	22.86 ± 1.09[a]	68.91 ± 0.78[bc]	48.52 ± 0.75[abcd]	20.38 ± 0.58[b]	40.21 ± 0.48[bcde]	8.32 ± 0.34[ab]
	305	Green	8.38 ± 0.86[a]	21.25 ± 1.26[a]	70.17 ± 0.97[abc]	46.04 ± 0.94[bcd]	24.31 ± 0.75[a]	38.88 ± 0.61[cde]	7.17 ± 0.41[b]
		Purple	8.93 ± 0.75[a]	22.85 ± 1.09[a]	68.22 ± 0.78[c]	45.11 ± 0.75[d]	23.11 ± 0.58[ab]	37.97 ± 0.48[e]	7.14 ± 0.34[ab]
	915	Green	7.86 ± 0.75[a]	20.31 ± 1.09[a]	71.83 ± 0.78[abc]	50.86 ± 0.75[a]	20.97 ± 0.58[ab]	43.16 ± 0.48[a]	7.70 ± 0.34[ab]
		Purple	8.03 ± 0.75[a]	20.69 ± 1.09[a]	71.28 ± 0.78[abc]	50.82 ± 0.75[ab]	20.46 ± 0.58[b]	42.47 ± 0.48[ab]	8.35 ± 0.34[ab]
2015	30	Green	9.16 ± 0.75[a]	19.57 ± 1.09[a]	71.27 ± 0.78[abc]	48.53 ± 0.75[abcd]	22.74 ± 0.58[ab]	40.85 ± 0.48[abcd]	7.68 ± 0.34[ab]
		Purple	8.56 ± 0.75[a]	22.92 ± 1.09[a]	68.52 ± 0.78[bc]	46.30 ± 0.75[cd]	22.22 ± 0.58[ab]	38.90 ± 0.48[de]	7.40 ± 0.34[ab]
	305	Green	8.32 ± 0.75[a]	18.81 ± 1.09[a]	72.86 ± 0.78[ab]	49.58 ± 0.75[abcd]	23.28 ± 0.58[ab]	40.53 ± 0.48[abcde]	9.05 ± 0.34[a]
		Purple	8.27 ± 0.75[a]	20.10 ± 1.09[a]	71.63 ± 0.78[abc]	48.58 ± 0.75[abcd]	23.05 ± 0.58[ab]	40.18 ± 0.48[bcde]	8.40 ± 0.34[ab]
	915	Green	7.30 ± 0.75[a]	20.08 ± 1.09[a]	72.61 ± 0.78[abc]	51.37 ± 0.75[a]	21.24 ± 0.58[ab]	42.93 ± 0.48[a]	8.44 ± 0.34[ab]
		Purple	6.25 ± 0.75[a]	21.58 ± 1.09[a]	72.17 ± 0.78[abc]	50.67 ± 0.75[ab]	21.50 ± 0.58[ab]	41.86 ± 0.48[abc]	8.80 ± 0.34[ab]

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.7. Least square means of composition of the Energycane and Napier grass collected across the three elevations and two harvest years (Unit: % dry matter)

Harvest year	Elevation (m)	Crop type	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
2014	30	Energycane	5.31 ± 0.50^{cd}	35.70 ± 1.13^{c}	59.00 ± 0.90^{b}	39.91 ± 0.81^{c}	19.09 ± 0.46^{ef}	33.00 ± 0.64^{cd}	6.91 ± 0.24^{cd}
		Napier grass	7.97 ± 0.50^{ab}	21.00 ± 1.13^{e}	71.03 ± 0.90^{a}	49.41 ± 0.81^{ab}	21.62 ± 0.46^{abcd}	41.28 ± 0.64^{ab}	8.13 ± 0.24^{abc}
	305	Energycane	8.78 ± 0.50^{a}	30.10 ± 1.13^{d}	61.12 ± 0.90^{b}	39.90 ± 0.81^{c}	21.22 ± 0.46^{bcde}	33.32 ± 0.64^{c}	6.58 ± 0.24^{de}
		Napier grass	8.75 ± 0.56^{a}	22.11 ± 1.26^{e}	69.00 ± 1.01^{a}	45.38 ± 0.90^{b}	23.63 ± 0.52^{a}	38.29 ± 0.71^{b}	7.11 ± 0.26^{cd}
	915	Energycane	3.78 ± 0.50^{d}	45.20 ± 1.13^{a}	51.03 ± 0.90^{d}	33.10 ± 0.81^{e}	17.93 ± 0.46^{f}	28.22 ± 0.64^{e}	4.87 ± 0.24^{f}
		Napier grass	7.95 ± 0.50^{ab}	20.50 ± 1.13^{e}	71.55 ± 0.90^{a}	50.84 ± 0.81^{a}	20.72 ± 0.46^{cde}	42.82 ± 0.64^{a}	8.02 ± 0.24^{abc}
2015	30	Energycane	5.44 ± 0.50^{cd}	36.38 ± 1.13^{c}	58.18 ± 0.90^{bc}	38.43 ± 0.81^{cd}	19.76 ± 0.46^{def}	32.08 ± 0.64^{cd}	6.35 ± 0.24^{de}
		Napier grass	8.86 ± 0.50^{a}	21.25 ± 1.13^{e}	69.89 ± 0.90^{a}	47.42 ± 0.81^{ab}	22.48 ± 0.46^{abc}	39.88 ± 0.64^{ab}	7.54 ± 0.24^{bcd}
	305	Energycane	5.91 ± 0.50^{bcd}	37.20 ± 1.13^{bc}	56.89 ± 0.90^{bc}	37.93 ± 0.81^{cd}	18.97 ± 0.46^{ef}	31.20 ± 0.64^{cde}	6.72 ± 0.24^{d}
		Napier grass	8.30 ± 0.50^{ab}	19.46 ± 1.13^{e}	72.25 ± 0.90^{a}	49.08 ± 0.81^{ab}	23.17 ± 0.46^{ab}	40.36 ± 0.64^{ab}	8.73 ± 0.24^{a}
	915	Energycane	3.72 ± 0.50^{d}	42.39 ± 1.13^{ab}	53.90 ± 0.90^{cd}	35.45 ± 0.81^{de}	18.44 ± 0.46^{f}	30.02 ± 0.64^{de}	5.43 ± 0.24^{ef}
		Napier grass	6.78 ± 0.50^{abc}	20.83 ± 1.13^{e}	72.39 ± 0.90^{a}	51.02 ± 0.81^{a}	21.37 ± 0.46^{abcd}	42.40 ± 0.64^{a}	8.62 ± 0.24^{ab}

*Least square mean values ± standard error (n = 6) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

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Table B.8. Least square means of composition of plant parts of the Energycane cultivars collected in 2015 across the three elevations (Unit: % dry matter)

Plant part	Elevation (m)	Cultivar	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
Leaf, Bottom	30	77-9271	10.33 ± 0.45^{bc}	19.02 ± 1.23^{ghi}	70.66 ± 1.02^{bcd}	44.48 ± 0.98^{bcdf}	26.18 ± 0.80^{cde}	38.19 ± 0.86^{abcd}	6.29 ± 0.26^{bcdefg}
		MOL 6136	12.98 ± 0.45^{a}	16.29 ± 1.23^{i}	70.73 ± 1.02^{bcd}	44.46 ± 0.98^{bdej}	26.27 ± 0.80^{cde}	38.19 ± 0.86^{abce}	6.26 ± 0.26^{bcdefg}
	305	77-9271	4.16 ± 0.45^{ij}	16.45 ± 1.23^{hi}	79.40 ± 1.02^{a}	50.16 ± 0.98^{a}	29.24 ± 0.80^{abc}	42.31 ± 0.86^{a}	7.85 ± 0.26^{a}
		MOL 6136	5.89 ± 0.45^{ghi}	15.90 ± 1.23^{i}	78.21 ± 1.02^{a}	50.00 ± 0.98^{a}	28.21 ± 0.80^{abcd}	42.43 ± 0.86^{a}	7.57 ± 0.26^{ab}
	915	77-9271	7.87 ± 0.45^{cdefg}	20.89 ± 1.23^{fghi}	71.24 ± 1.02^{bcd}	44.85 ± 0.98^{abceg}	26.39 ± 0.80^{cde}	38.09 ± 0.86^{abcde}	6.76 ± 0.26^{abcd}
		MOL 6136	8.68 ± 0.45^{cdef}	19.28 ± 1.23^{ghi}	72.03 ± 1.02^{b}	45.57 ± 0.98^{ab}	26.46 ± 0.80^{cde}	39.18 ± 0.86^{ab}	6.39 ± 0.26^{bcdef}
Leaf, Top	30	77-9271	8.69 ± 0.45^{cdef}	22.57 ± 1.23^{fghi}	68.74 ± 1.02^{bcde}	39.56 ± 0.98^{eghijk}	29.18 ± 0.80^{abc}	34.85 ± 0.86^{bcdef}	4.71 ± 0.26^{hij}
		MOL 6136	11.75 ± 0.45^{ab}	20.54 ± 1.23^{fghi}	67.71 ± 1.02^{bcdef}	39.31 ± 0.98^{fhik}	28.40 ± 0.80^{abcd}	34.18 ± 0.86^{dfg}	5.13 ± 0.26^{efghij}
	305	77-9271	4.49 ± 0.45^{hij}	23.20 ± 1.23^{efg}	72.31 ± 1.02^{bc}	41.37 ± 0.98^{bcdefghj}	30.94 ± 0.80^{ab}	35.35 ± 0.86^{bcdef}	6.03 ± 0.26^{cdefgh}
		MOL 6136	6.23 ± 0.45^{fghi}	23.21 ± 1.23^{defgh}	70.56 ± 1.02^{bcd}	38.67 ± 0.98^{hikl}	31.89 ± 0.80^{a}	33.98 ± 0.86^{cdefg}	4.70 ± 0.26^{hij}
	915	77-9271	7.92 ± 0.45^{cdefg}	23.63 ± 1.23^{defg}	68.45 ± 1.02^{bcdef}	41.63 ± 0.98^{bcdefghj}	26.83 ± 0.80^{bcde}	36.40 ± 0.86^{bcde}	5.22 ± 0.26^{efghij}
		MOL 6136	9.02 ± 0.45^{cd}	24.14 ± 1.23^{defg}	66.83 ± 1.02^{cdef}	38.46 ± 0.98^{hikl}	28.38 ± 0.80^{abcd}	33.53 ± 0.86^{cdefg}	4.93 ± 0.26^{ghij}

Table B.8. (Continued) Least square means of composition of plant parts of the Energycane cultivars collected in 2015 across the three elevations (Unit: % dry matter)

Plant part	Elevation (m)	Cultivar	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
Stem, Bottom	30	77-9271	2.40 ± 0.45^{jk}	45.10 ± 1.23^{ab}	52.50 ± 1.02^{jk}	35.81 ± 0.98^{iklm}	16.70 ± 0.80^{gh}	29.26 ± 0.86^{gh}	6.55 ± 0.26^{abcde}
		MOL 6136	3.99 ± 0.45^{hij}	41.13 ± 1.23^{b}	54.89 ± 1.02^{ij}	38.38 ± 0.98^{hikl}	16.51 ± 0.80^{h}	31.51 ± 0.86^{fgh}	6.87 ± 0.26^{abcd}
	305	77-9271	1.13 ± 0.45^{k}	49.00 ± 1.23^{a}	49.87 ± 1.02^{jk}	34.90 ± 0.98^{iklm}	14.97 ± 0.80^{h}	27.97 ± 0.86^{h}	6.93 ± 0.26^{abc}
		MOL 6136	2.15 ± 0.45^{jk}	48.37 ± 1.23^{a}	49.48 ± 1.02^{jk}	35.08 ± 0.98^{klm}	14.40 ± 0.80^{h}	28.36 ± 0.86^{h}	6.73 ± 0.26^{abcd}
	915	77-9271	1.47 ± 0.45^{k}	50.66 ± 1.23^{a}	47.87 ± 1.02^{k}	33.38 ± 0.98^{lm}	14.49 ± 0.80^{h}	27.88 ± 0.86^{h}	5.49 ± 0.26^{defghi}
		MOL 6136	2.17 ± 0.45^{jk}	48.69 ± 1.23^{a}	49.13 ± 1.02^{k}	32.57 ± 0.98^{m}	16.57 ± 0.80^{gh}	27.03 ± 0.86^{h}	5.53 ± 0.26^{cdefgh}
Stem, Top	30	77-9271	7.89 ± 0.45^{defg}	28.84 ± 1.23^{cde}	63.26 ± 1.02^{fgh}	38.72 ± 0.98^{hijkl}	24.55 ± 0.80^{def}	33.91 ± 0.86^{ef}	4.82 ± 0.26^{hij}
		MOL 6136	9.35 ± 0.45^{cd}	24.49 ± 1.23^{defg}	66.16 ± 1.02^{defg}	40.05 ± 0.98^{cfghik}	26.11 ± 0.80^{cde}	34.83 ± 0.86^{bcdef}	5.23 ± 0.26^{efghij}
	305	77-9271	6.37 ± 0.45^{efgh}	29.47 ± 1.23^{cd}	64.16 ± 1.02^{efgh}	38.82 ± 0.98^{hikl}	25.35 ± 0.80^{cdef}	34.00 ± 0.86^{cdefg}	4.81 ± 0.26^{hij}
		MOL 6136	7.67 ± 0.45^{defg}	26.55 ± 1.23^{cdef}	65.78 ± 1.02^{defgh}	39.22 ± 0.98^{dfhij}	26.56 ± 0.80^{bcde}	34.23 ± 0.86^{cdef}	4.99 ± 0.26^{fghij}
	915	77-9271	7.59 ± 0.45d^{efg}	31.93 ± 1.23^{c}	60.48 ± 1.02^{hi}	39.35 ± 0.98^{dfhijk}	21.12 ± 0.80^{fg}	35.34 ± 0.86^{bcdef}	4.02 ± 0.26^{j}
		MOL 6136	8.72 ± 0.45^{cde}	29.91 ± 1.23^{cde}	61.36 ± 1.02^{gh}	38.97 ± 0.98^{ghikl}	22.39 ± 0.80^{ef}	34.82 ± 0.86^{cdef}	4.15 ± 0.26^{ij}

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.9. Least square means of composition of the Energycane cultivars collected in 2015 across the three elevations (Unit: % dry matter)

Cultivar	Elevation (m)	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
77-9271	30	4.25 ± 0.54^{ab}	38.94 ± 1.40^{ab}	56.81 ± 1.08^{a}	37.33 ± 1.06^{a}	19.48 ± 0.71^{a}	31.10 ± 0.91^{a}	6.23 ± 0.21^{ab}
	305	5.65 ± 0.54^{ab}	37.47 ± 1.40^{ab}	56.88 ± 1.08^{a}	37.89 ± 1.06^{a}	18.98 ± 0.71^{a}	31.01 ± 0.91^{a}	6.88 ± 0.21^{a}
	915	3.46 ± 0.54^{b}	42.69 ± 1.40^{a}	53.84 ± 1.08^{a}	36.08 ± 1.06^{a}	17.76 ± 0.71^{a}	30.64 ± 0.91^{a}	5.44 ± 0.21^{b}
MOL 6136	30	6.63 ± 0.54^{a}	33.82 ± 1.40^{b}	59.55 ± 1.08^{a}	39.53 ± 1.06^{a}	20.03 ± 0.71^{a}	33.05 ± 0.91^{a}	6.47 ± 0.21^{ab}
	305	6.16 ± 0.54^{ab}	36.94 ± 1.40^{ab}	56.90 ± 1.08^{a}	37.96 ± 1.06^{a}	18.95 ± 0.71^{a}	31.40 ± 0.91^{a}	6.55 ± 0.21^{ab}
	915	3.98 ± 0.54^{ab}	42.08 ± 1.40^{a}	53.95 ± 1.08^{a}	34.83 ± 1.06^{a}	19.12 ± 0.71^{a}	29.40 ± 0.91^{a}	5.42 ± 0.21^{b}

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.10. Least square means of composition of plant parts of Napier grass cultivars collected in two seasons of 2015 across the three elevations (Unit: % dry matter)

Harvest season	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
March	30	Green	Leaf	7.08 ± 1.24^{cdef}	27.66 ± 1.45^{abcd}	65.26 ± 1.15^{ijklm}	38.04 ± 1.16^{d}	27.22 ± 0.97^{abc}	34.38 ± 0.82^{gh}	3.66 ± 0.46^{d}
			Stem	15.32 ± 1.24^{ab}	12.94 ± 1.45^{jk}	71.74 ± 1.15^{defgh}	52.29 ± 1.16^{c}	19.44 ± 0.97^{hij}	42.18 ± 0.82^{cd}	10.12 ± 0.46^{abc}
		Purple	Leaf	7.83 ± 1.24^{cdef}	27.19 ± 1.45^{abcd}	64.99 ± 1.15^{jlm}	38.44 ± 1.16^{d}	26.54 ± 0.97^{abcdf}	34.59 ± 0.82^{gh}	3.85 ± 0.46^{d}
			Stem	15.96 ± 1.24^{a}	13.71 ± 1.45^{hk}	$70.34 \pm 1.15^{defghijk}$	48.89 ± 1.16^{c}	21.44 ± 0.97^{eghij}	40.43 ± 0.82^{cdef}	8.47 ± 0.46^{bc}
	305	Green	Leaf	5.88 ± 1.24^{def}	27.33 ± 1.45^{abcd}	66.79 ± 1.15^{gklm}	40.56 ± 1.16^{d}	26.24 ± 0.97^{abcdef}	35.54 ± 0.82^{gh}	5.02 ± 0.46^{d}
			Stem	8.43 ± 1.24^{bcdef}	18.21 ± 1.45^{efghijk}	73.36 ± 1.15^{abcde}	52.92 ± 1.16^{c}	20.44 ± 0.97^{ghij}	41.95 ± 0.82^{cde}	10.97 ± 0.46^{ab}
		Purple	Leaf	3.68 ± 1.24^{ef}	31.22 ± 1.45^{a}	65.10 ± 1.15^{klm}	38.51 ± 1.16^{d}	26.60 ± 0.97^{abcdef}	34.07 ± 0.82^{gh}	4.43 ± 0.46^{d}
			Stem	7.71 ± 1.24^{cdef}	19.56 ± 1.45^{dfghij}	72.74 ± 1.15^{bcdef}	51.87 ± 1.16^{c}	20.87 ± 0.97^{ghij}	41.57 ± 0.82^{cde}	10.29 ± 0.46^{abc}
	915	Green	Leaf	15.30 ± 1.24^{ab}	22.34 ± 1.45^{bcdefg}	62.36 ± 1.15^{m}	35.33 ± 1.16^{d}	27.02 ± 0.97^{abcde}	31.31 ± 0.82^{h}	4.03 ± 0.46^{d}
			Stem	5.91 ± 1.24^{def}	22.76 ± 1.45^{bcdefg}	$71.33 \pm 1.15^{cdefghijk}$	50.02 ± 1.16^{c}	21.31 ± 0.97^{fghij}	42.02 ± 0.82^{cd}	8.00 ± 0.46^{c}
		Purple	Leaf	11.70 ± 1.24^{abcd}	25.29 ± 1.45^{abcdef}	63.01 ± 1.15^{lm}	35.70 ± 1.16^{d}	27.31 ± 0.97^{abcd}	31.30 ± 0.82^{h}	4.41 ± 0.46^{d}
			Stem	4.15 ± 1.24^{ef}	24.12 ± 1.45^{abcdefg}	71.73 ± 1.15^{cdefgik}	49.05 ± 1.16^{c}	$22.68 \pm 0.97^{cdefghij}$	41.01 ± 0.82^{cde}	8.03 ± 0.46^{c}

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.10. (Continued) Least square means of composition of plant parts of Napier grass cultivars collected in two seasons of 2015 across the three elevations (Unit: % dry matter)

Harvest season	Elevation (m)	Cultivar	Plant part	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
Sept.	30	Green	Leaf	3.56 ± 1.24^{ef}	30.29 ± 1.45^{ab}	66.15 ± 1.15^{fghijklm}	41.86 ± 1.16^{d}	24.29 ± 0.97^{abcdefgh}	37.89 ± 0.82^{defg}	3.97 ± 0.46^{d}
			Stem	10.26 ± 1.24^{abcde}	12.79 ± 1.45^{jk}	76.96 ± 1.15^{abc}	54.74 ± 1.16^{bc}	22.22 ± 0.97^{defghij}	44.54 ± 0.82^{bc}	10.20 ± 0.46^{abc}
		Purple	Leaf	4.48 ± 1.24^{ef}	31.47 ± 1.45^{a}	64.05 ± 1.15^{lm}	40.21 ± 1.16^{d}	23.84 ± 0.97^{bcdefghi}	35.77 ± 0.82^{fgh}	4.44 ± 0.46^{d}
			Stem	6.91 ± 1.24^{cdef}	21.32 ± 1.45^{cdefgij}	71.78 ± 1.15^{cdefgh}	51.80 ± 1.16^{c}	19.98 ± 0.97^{ghij}	41.66 ± 0.82^{cde}	10.13 ± 0.46^{abc}
	305	Green	Leaf	7.31 ± 1.24^{cdef}	21.30 ± 1.45^{cdefgh}	71.39 ± 1.15^{cdefhij}	41.68 ± 1.16^{d}	29.71 ± 0.97^{a}	37.09 ± 0.82^{efg}	4.59 ± 0.46^{d}
			Stem	10.20 ± 1.24^{abcde}	13.41 ± 1.45^{ijk}	76.38 ± 1.15^{abcd}	54.11 ± 1.16^{bc}	22.28 ± 0.97^{cdefghij}	43.15 ± 0.82^{c}	10.96 ± 0.46^{ab}
		Purple	Leaf	4.23 ± 1.24^{ef}	26.72 ± 1.45^{abce}	69.06 ± 1.15^{efghijkl}	40.49 ± 1.16^{d}	28.57 ± 0.97^{ab}	35.87 ± 0.82^{fgh}	4.62 ± 0.46^{d}
			Stem	13.91 ± 1.24^{abc}	11.39 ± 1.45^{k}	74.71 ± 1.15^{abcde}	54.05 ± 1.16^{bc}	20.66 ± 0.97^{ghij}	43.88 ± 0.82^{c}	10.16 ± 0.46^{abc}
	915	Green	Leaf	12.52 ± 1.24^{abcd}	21.68 ± 1.45^{cdefghi}	65.81 ± 1.15^{hjlm}	40.51 ± 1.16^{d}	25.29 ± 0.97^{abcdefg}	35.58 ± 0.82^{gh}	4.94 ± 0.46^{d}
			Stem	3.64 ± 1.24^{ef}	17.43 ± 1.45^{fghijk}	78.92 ± 1.15^{ab}	60.63 ± 1.16^{ab}	18.29 ± 0.97^{ij}	49.41 ± 0.82^{a}	11.22 ± 0.46^{a}
		Purple	Leaf	12.70 ± 1.24^{abcd}	24.53 ± 1.45^{abcdef}	62.77 ± 1.15^{lm}	38.61 ± 1.16^{d}	24.16 ± 0.97^{abcdefgh}	33.97 ± 0.82^{gh}	4.65 ± 0.46^{d}
			Stem	2.74 ± 1.24^{f}	17.69 ± 1.45^{ghijk}	79.58 ± 1.15^{a}	61.74 ± 1.16^{a}	17.83 ± 0.97^{j}	49.24 ± 0.82^{ab}	12.50 ± 0.46^{a}

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.11. Least square means of composition of the Napier grass cultivars collected in 2015 across the three elevations (Unit: % dry matter)

Cultivar	Elevation (m)	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
Green	30	9.16 ± 0.62[a]	19.57 ± 1.09[a]	71.27 ± 0.92[a]	48.53 ± 0.79[ab]	22.74 ± 0.61[a]	40.85 ± 0.49[abc]	7.68 ± 0.36[a]
	305	8.32 ± 0.62[a]	18.81 ± 1.09[a]	72.86 ± 0.92[a]	49.58 ± 0.79[ab]	23.28 ± 0.61[a]	40.53 ± 0.49[abc]	9.05 ± 0.36[a]
	915	7.30 ± 0.62[a]	20.08 ± 1.09[a]	72.61 ± 0.92[a]	51.37 ± 0.79[a]	21.24 ± 0.61[a]	42.93 ± 0.49[a]	8.44 ± 0.36[a]
Purple	30	8.56 ± 0.62[a]	22.92 ± 1.09[a]	68.52 ± 0.92[a]	46.30 ± 0.79[b]	22.22 ± 0.61[a]	38.90 ± 0.49[c]	7.40 ± 0.36[a]
	305	8.27 ± 0.62[a]	20.10 ± 1.09[a]	71.63 ± 0.92[a]	48.58 ± 0.79[ab]	23.05 ± 0.61[a]	40.18 ± 0.49[bc]	8.40 ± 0.36[a]
	915	6.25 ± 0.62[a]	21.58 ± 1.09[a]	72.17 ± 0.92[a]	50.67 ± 0.79[ab]	21.50 ± 0.61[a]	41.86 ± 0.49[ab]	8.80 ± 0.36[a]

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table B.12. Least square means of composition of the Energycane and Napier grass collected in 2015 across the three elevations (Unit: % dry matter)

Crop type	Elevation (m)	Ash	Ash free extractives	NDF	ADF	Hemicellulose	Cellulose	Lignin (ADL)
Energycane	30	5.44 ± 0.39^{bc}	36.38 ± 0.84^{b}	58.18 ± 0.77^{b}	38.43 ± 0.57^{c}	19.76 ± 0.50^{bc}	32.08 ± 0.46^{b}	6.35 ± 0.20^{cd}
	305	5.91 ± 0.39^{bc}	37.20 ± 0.84^{b}	56.89 ± 0.77^{b}	37.93 ± 0.57^{c}	18.97 ± 0.50^{bc}	31.20 ± 0.46^{b}	6.72 ± 0.20^{c}
	915	3.72 ± 0.39^{c}	42.39 ± 0.84^{a}	53.90 ± 0.77^{b}	35.45 ± 0.57^{c}	18.44 ± 0.50^{c}	30.02 ± 0.46^{b}	5.43 ± 0.20^{d}
Napier grass	30	8.86 ± 0.39^{a}	21.25 ± 0.84^{c}	69.89 ± 0.77^{a}	47.42 ± 0.57^{b}	22.48 ± 0.50^{a}	39.88 ± 0.46^{a}	7.54 ± 0.20^{bc}
	305	8.30 ± 0.39^{a}	19.46 ± 0.84^{c}	72.25 ± 0.77^{a}	49.08 ± 0.57^{ab}	23.17 ± 0.50^{a}	40.36 ± 0.46^{a}	8.73 ± 0.20^{a}
	915	6.78 ± 0.39^{ab}	20.83 ± 0.84^{c}	72.39 ± 0.77^{a}	51.02 ± 0.57^{a}	21.37 ± 0.50^{ab}	42.40 ± 0.46^{a}	8.62 ± 0.20^{ab}

*Least square mean values ± standard error (n = 6) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

APPENDIX C: ANAEROBIC DIGESTION DATA OF ENERGY CROPS

Table C.1. Least square means of methane yields of plant parts of the Energycane cultivars collected in 2015 across the three elevations

Plant part	Elevation (m)	Cultivar	Methane content (%)	Specific methane yield [Nm^3 (kg VS_{added})$^{-1}$]	Total methane yield (Nm^3 ha^{-1} $year^{-1}$)
Leaf, Bottom	30	77-9271	54.90 ± 0.47^{cdefghijl}	0.214 ± 0.008^{efghi}	497.01 ± 563.12^{e}
		MOL 6136	56.56 ± 0.47^{abcde}	0.194 ± 0.008^{gi}	572.44 ± 563.12^{e}
	305	77-9271	57.47 ± 0.47^{abc}	0.182 ± 0.008^{i}	1,007.45 ± 563.12^{de}
		MOL 6136	55.76 ± 0.47^{cdefh}	0.192 ± 0.008^{hi}	1,208.26 ± 563.12^{de}
	915	77-9271	59.04 ± 0.47^{a}	0.194 ± 0.008^{fi}	775.88 ± 563.12^{de}
		MOL 6136	55.57 ± 0.47^{cdefg}	0.219 ± 0.008^{defghi}	915.16 ± 563.12^{de}
Leaf, Top	30	77-9271	55.60 ± 0.47^{cdefgh}	0.254 ± 0.008^{abcd}	703.35 ± 563.12^{de}
		MOL 6136	55.39 ± 0.47^{cdefghi}	0.260 ± 0.008^{abc}	686.11 ± 563.12^{de}
	305	77-9271	56.41 ± 0.47^{bcd}	0.255 ± 0.008^{abcde}	901.32 ± 563.12^{de}
		MOL 6136	54.98 ± 0.47^{cdefghijk}	0.258 ± 0.008^{abcd}	1,075.86 ± 563.12^{de}
	915	77-9271	58.51 ± 0.47^{ab}	0.229 ± 0.008^{cdegh}	1,331.19 ± 563.12^{de}
		MOL 6136	55.48 ± 0.47^{cdefg}	0.251 ± 0.008^{abcde}	1,325.46 ± 563.12d^{e}
Stem, Bottom	30	77-9271	52.65 ± 0.47^{km}	0.219 ± 0.008^{defghi}	4,467.83 ± 563.12^{c}
		MOL 6136	52.47 ± 0.47^{jklm}	0.230 ± 0.008^{cdefh}	3,488.66 ± 563.12^{cd}
	305	77-9271	52.87 ± 0.47^{ijklm}	0.229 ± 0.008^{cdefg}	6,277.74 ± 563.12^{bc}
		MOL 6136	53.21 ± 0.47^{gijklm}	0.223 ± 0.008^{cdefgh}	6,195.56 ± 563.12^{bc}
	915	77-9271	52.35 ± 0.47^{lm}	0.239 ± 0.008^{abcde}	7,726.52 ± 563.12^{ab}
		MOL 6136	52.08 ± 0.47^{m}	0.239 ± 0.008^{bcde}	9,750.58 ± 563.12^{a}
Stem, Top	30	77-9271	52.42 ± 0.47^{km}	0.252 ± 0.008^{abcd}	372.12 ± 563.12^{e}
		MOL 6136	53.60 ± 0.47^{fghijklm}	0.251 ± 0.008^{abcd}	303.22 ± 563.12^{e}
	305	77-9271	54.04 ± 0.47^{efghijklm}	0.246 ± 0.008^{abcde}	334.59 ± 563.12^{e}
		MOL 6136	54.17 ± 0.47^{defghijklm}	0.247 ± 0.008^{abcde}	395.91 ± 563.12^{de}
	915	77-9271	53.76 ± 0.47^{fghijklm}	0.273 ± 0.008^{a}	1,033.64 ± 563.12^{de}
		MOL 6136	53.23 ± 0.47^{hijklm}	0.271 ± 0.008^{ab}	1,149.53 ± 563.12^{de}

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table C.2. Least square means of methane yields from Energycane cultivars collected in 2015 across the three elevations

Cultivar	Elevation (m)	Methane content (%)	Specific methane yield [Nm^3 (kg VS_{added})$^{-1}$]	Total methane yield (Nm^3 ha^{-1} $year^{-1}$)
77-9271	30	53.16 ± 0.43[a]	0.223 ± 0.004[a]	6,040.30 ± 1,370.47[ab]
	305	53.77 ± 0.43[a]	0.225 ± 0.004[a]	8,521.10 ± 1,370.47[ab]
	915	53.60 ± 0.43[a]	0.237 ± 0.004[a]	10,867.23 ± 1,370.47[ab]
MOL 6136	30	53.37 ± 0.43[a]	0.230 ± 0.004[a]	5,050.44 ± 1,370.47[b]
	305	53.78 ± 0.43[a]	0.223 ± 0.004[a]	8,875.59 ± 1,370.47[ab]
	915	52.76 ± 0.43[a]	0.241 ± 0.004[a]	13,140.73 ± 1,370.47[a]

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table C.3. Least square means of methane yields from plant parts of Napier grass cultivars collected in two seasons of 2015 across the three elevations

Harvest season	Elevation (m)	Cultivar	Plant part	Methane content (%)	Specific methane yield [Nm^3 (kg VS_{added})$^{-1}$]	Total methane yield (Nm^3 ha^{-1} $year^{-1}$)
March	30	Green	Leaf	54.92 ± 0.60^{abc}	0.263 ± 0.008^{a}	724.54 ± 240.11^{ef}
			Stem	57.10 ± 0.60^{ab}	0.162 ± 0.008^{def}	849.84 ± 240.11^{ef}
		Purple	Leaf	56.76 ± 0.60^{abc}	0.241 ± 0.008^{ab}	606.31 ± 240.11^{f}
			Stem	55.64 ± 0.60^{abc}	0.186 ± 0.008^{cdef}	619.99 ± 240.11^{f}
	305	Green	Leaf	56.32 ± 0.60^{abc}	0.241 ± 0.008^{ab}	596.35 ± 240.11^{f}
			Stem	58.26 ± 0.60^{a}	0.151 ± 0.008^{f}	1,263.10 ± 240.11^{cdef}
		Purple	Leaf	56.35 ± 0.60^{abc}	0.255 ± 0.008^{a}	702.84 ± 240.11^{ef}
			Stem	55.57 ± 0.60^{abc}	0.200 ± 0.008^{bcde}	1,663.52 ± 240.11^{bcdef}
	915	Green	Leaf	55.00 ± 0.60^{abc}	0.271 ± 0.008^{a}	1,353.90 ± 240.11^{ef}
			Stem	53.38 ± 0.60^{c}	0.204 ± 0.008^{bcd}	2,360.07 ± 240.11^{bcd}
		Purple	Leaf	54.38 ± 0.60^{bc}	0.238 ± 0.008^{ab}	1,344.46 ± 240.11^{cdef}
			Stem	54.42 ± 0.60^{bc}	0.158 ± 0.008^{ef}	1,815.81 ± 240.11^{bcdef}
Sept.	30	Green	Leaf	54.83 ± 0.60^{abc}	0.235 ± 0.008^{ab}	1,366.17 ± 240.11^{cdef}
			Stem	57.53 ± 0.60^{ab}	0.161 ± 0.008^{def}	990.69 ± 240.11^{def}
		Purple	Leaf	55.78 ± 0.60^{abc}	0.233 ± 0.008^{ab}	975.12 ± 240.11^{ef}
			Stem	56.20 ± 0.60^{abc}	0.162 ± 0.008^{def}	998.66 ± 240.11^{def}
	305	Green	Leaf	55.72 ± 0.60^{abc}	0.240 ± 0.008^{ab}	787.79 ± 240.11^{ef}
			Stem	57.24 ± 0.60^{ab}	0.155 ± 0.008^{ef}	691.44 ± 240.11^{ef}
		Purple	Leaf	54.73 ± 0.60^{bc}	0.230 ± 0.008^{abc}	1,044.71 ± 240.11^{def}
			Stem	56.35 ± 0.60^{abc}	0.161 ± 0.008^{def}	963.14 ± 240.11^{ef}
	915	Green	Leaf	55.58 ± 0.60^{abc}	0.242 ± 0.008^{ab}	2,633.12 ± 240.11^{bc}
			Stem	56.57 ± 0.60^{abc}	0.165 ± 0.008^{def}	4,075.59 ± 240.11^{a}
		Purple	Leaf	54.92 ± 0.60^{abc}	0.232 ± 0.008^{ab}	2,023.38 ± 240.11^{cde}
			Stem	56.21 ± 0.60^{abc}	0.146 ± 0.008^{f}	3,001.53 ± 240.11^{ab}

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table C.4. Least square means of methane yields from Napier grass cultivars collected in 2015 across the three elevations

Cultivar	Elevation (m)	Methane content (%)	Specific methane yield [Nm^3 $(kg\ VS_{added})^{-1}$]	Total methane yield ($Nm^3\ ha^{-1}\ year^{-1}$)
Green	30	56.00 ± 0.31[ab]	0.196 ± 0.006[a]	3,931.24 ± 718.67[c]
	305	57.01 ± 0.31[a]	0.180 ± 0.006[a]	3,338.67 ± 718.67[c]
	915	55.40 ± 0.31[ab]	0.199 ± 0.006[a]	10,422.69 ± 718.67[a]
Purple	30	56.01 ± 0.31[ab]	0.197 ± 0.006[a]	3,200.08 ± 718.67[c]
	305	55.69 ± 0.31[ab]	0.202 ± 0.006[a]	4,374.21 ± 718.67[bc]
	915	55.18 ± 0.31[b]	0.176 ± 0.006[a]	8,185.18 ± 718.67[ab]

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table C.5. Least square means of methane yields from Energycane and Napier grass collected in 2015 across the three elevations

Crop type	Elevation (m)	Methane content (%)	Specific methane yield [Nm^3 $(kg\ VS_{added})^{-1}$]	Total methane yield ($Nm^3\ ha^{-1}\ year^{-1}$)
Energycane	30	53.26 ± 0.26[b]	0.227 ± 0.004[a]	5,545.37 ± 863.78[bc]
	305	53.78 ± 0.26[b]	0.224 ± 0.004[a]	8,698.35 ± 863.78[ab]
	915	53.18 ± 0.26[b]	0.239 ± 0.004[a]	12,003.98 ± 863.78[a]
Napier grass	30	56.01 ± 0.26[a]	0.197 ± 0.004[b]	3,565.66 ± 863.78[c]
	305	56.35 ± 0.26[a]	0.191 ± 0.004[b]	3,856.44 ± 863.78[c]
	915	55.29 ± 0.26[a]	0.187 ± 0.004[b]	9,303.93 ± 863.78[ab]

*Least square mean values ± standard error (n = 6) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table C.6. Least square means of specific methane yield of selected samples of plant parts of the Energycane at different incubation time

Plant parts	Incubation time (days)	Specific methane yield [Nm^3 (kg VS_{added})$^{-1}$]
Leaf, Bottom	15	0.144 ± 0.003^{n}
	30	0.198 ± 0.003^{l}
	35	0.206 ± 0.003^{kl}
	45	0.206 ± 0.003^{kl}
	60	0.218 ± 0.003^{ijk}
	75	0.226 ± 0.003^{hij}
	90	0.240 ± 0.003^{fgh}
Leaf, Top	15	0.172 ± 0.003^{m}
	30	0.234 ± 0.003^{ghi}
	35	0.240 ± 0.003^{fgh}
	45	0.240 ± 0.003^{fgh}
	60	0.253 ± 0.003^{def}
	75	0.261 ± 0.003^{cde}
	90	0.274 ± 0.003^{bc}
Stem, Bottom	15	0.209 ± 0.003^{jkl}
	30	0.236 ± 0.003^{fghi}
	35	0.239 ± 0.003^{fgh}
	45	0.239 ± 0.003^{fgh}
	60	0.244 ± 0.003^{efgh}
	75	0.248 ± 0.003^{efg}
	90	0.253 ± 0.003^{def}
Stem, Top	15	0.231 ± 0.003^{ghi}
	30	0.269 ± 0.003^{bcd}
	35	0.272 ± 0.003^{bc}
	45	0.272 ± 0.003^{bc}
	60	0.280 ± 0.003^{ab}
	75	0.285 ± 0.003^{ab}
	90	0.294 ± 0.003^{a}

*Least square mean values ± standard error (n = 6) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table C.7. Least square means of specific methane yield of selected samples of plant parts of the Energycane cultivars at different incubation time

Plant part	Incubation time (days)	Cultivar	Specific methane yield [Nm^3 (kg VS_{added})$^{-1}$]
Leaf, Bottom	15	77-9271	0.126 ± 0.005^{w}
		MOL 6136	0.162 ± 0.005^{uv}
	30	77-9271	0.183 ± 0.005^{tuv}
		MOL 6136	0.213 ± 0.005^{mnopqrs}
	35	77-9271	0.194 ± 0.005^{rst}
		MOL 6136	0.219 ± 0.005^{lmnopqr}
	45	77-9271	0.194 ± 0.005^{rst}
		MOL 6136	0.219 ± 0.005^{lmnopqr}
	60	77-9271	0.204 ± 0.005^{qrst}
		MOL 6136	0.231 ± 0.005^{ijklmnop}
	75	77-9271	0.213 ± 0.005^{nopqrs}
		MOL 6136	0.240 ± 0.005^{hijklm}
	90	77-9271	0.226 ± 0.005^{jklmnopq}
		MOL 6136	0.254 ± 0.005^{cdefghi}
Leaf, Top	15	77-9271	0.157 ± 0.005^{v}
		MOL 6136	0.187 ± 0.005^{stu}
	30	77-9271	0.223 ± 0.005^{klmnopq}
		MOL 6136	0.245 ± 0.005^{efghijkl}
	35	77-9271	0.229 ± 0.005^{ijklmnopq}
		MOL 6136	0.251 ± 0.005^{defghij}
	45	77-9271	0.229 ± 0.005^{ijklmnopq}
		MOL 6136	0.251 ± 0.005^{defghij}
	60	77-9271	0.243 ± 0.005^{ghijkl}
		MOL 6136	0.263 ± 0.005^{bcdefgh}
	75	77-9271	0.250 ± 0.005^{defghij}
		MOL 6136	0.271 ± 0.005^{abcdef}
	90	77-9271	0.263 ± 0.005^{bcdefgh}
		MOL 6136	0.285 ± 0.005^{ab}

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table C.7. (Continued) Least square means of specific methane yield of selected samples of plant parts of the Energycane cultivars at different incubation time

Plant part	Incubation time (days)	Cultivar	Specific methane yield [Nm^3 (kg VS_{added})$^{-1}$]
Stem, Bottom	15	77-9271	0.207 ± 0.005^{pqrst}
		MOL 6136	0.211 ± 0.005^{opqrs}
	30	77-9271	0.235 ± 0.005^{ijklmno}
		MOL 6136	0.236 ± 0.005^{hijklmno}
	35	77-9271	0.238 ± 0.005^{hijklmn}
		MOL 6136	0.239 ± 0.005^{hijklmn}
	45	77-9271	0.238 ± 0.005^{hijklmn}
		MOL 6136	0.239 ± 0.005^{hijklmn}
	60	77-9271	0.245 ± 0.005^{efghijkl}
		MOL 6136	0.244 ± 0.005^{fghijkl}
	75	77-9271	0.248 ± 0.005^{defghijk}
		MOL 6136	0.247 ± 0.005^{defghijk}
	90	77-9271	0.253 ± 0.005^{cdefghij}
		MOL 6136	0.254 ± 0.005^{cdefghi}
Stem, Top	15	77-9271	0.231 ± 0.005^{ijklmnopq}
		MOL 6136	0.231 ± 0.005^{ijklmnopq}
	30	77-9271	0.270 ± 0.005^{abcdefg}
		MOL 6136	0.268 ± 0.005^{abcdefg}
	35	77-9271	0.273 ± 0.005^{abcd}
		MOL 6136	0.272 ± 0.005^{abcde}
	45	77-9271	0.273 ± 0.005^{abcd}
		MOL 6136	0.272 ± 0.005^{abcde}
	60	77-9271	0.280 ± 0.005^{abc}
		MOL 6136	0.280 ± 0.005^{abc}
	75	77-9271	0.284 ± 0.005^{ab}
		MOL 6136	0.285 ± 0.005^{ab}
	90	77-9271	0.292 ± 0.005^{a}
		MOL 6136	0.295 ± 0.005^{a}

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table C.8. Least square means of specific methane yield of selected samples of plant parts of the Napier grass at different incubation time

Plant part	Incubation time (days)	Specific methane yield [Nm^3 (kg VS_{added})$^{-1}$]
Leaf	15	0.180 ± 0.004^{fghi}
	30	0.236 ± 0.004^{e}
	35	0.244 ± 0.004^{de}
	45	0.255 ± 0.004^{cd}
	60	0.266 ± 0.004^{bc}
	75	0.275 ± 0.004^{ab}
	90	0.286 ± 0.004^{a}
Stem	15	0.128 ± 0.004^{j}
	30	0.166 ± 0.004^{i}
	35	0.170 ± 0.004^{hi}
	45	0.178 ± 0.004g^{hi}
	60	0.185 ± 0.004^{fgh}
	75	0.191 ± 0.004^{fg}
	90	0.197 ± 0.004^{f}

*Least square mean values ± standard error (n = 21) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table C.9. Least square means of specific methane yield of selected samples of plant parts of the Napier grass cultivars at different incubation time

Plant part	Incubation time (days)	Cultivar	Specific methane yield [Nm^3 ($kg\ VS_{added}$)$^{-1}$]
Leaf	15	Green	0.183 ± 0.006[hijk]
		Purple	0.177 ± 0.005[ijk]
	30	Green	0.242 ± 0.006[def]
		Purple	0.230 ± 0.005[fg]
	35	Green	0.251 ± 0.006[cdef]
		Purple	0.238 ± 0.005[ef]
	45	Green	0.264 ± 0.006[bcde]
		Purple	0.247 ± 0.005[cdef]
	60	Green	0.274 ± 0.006[abc]
		Purple	0.258 ± 0.005[bcde]
	75	Green	0.284 ± 0.006[ab]
		Purple	0.267 ± 0.005[abcd]
	90	Green	0.293 ± 0.006[a]
		Purple	0.279 ± 0.005[ab]
Stem	15	Green	0.124 ± 0.006[l]
		Purple	0.132 ± 0.005[l]
	30	Green	0.170 ± 0.006[ijk]
		Purple	0.162 ± 0.005[k]
	35	Green	0.175 ± 0.006[ijk]
		Purple	0.166 ± 0.005[jk]
	45	Green	0.185 ± 0.006[hijk]
		Purple	0.171 ± 0.005[jk]
	60	Green	0.192 ± 0.006[hij]
		Purple	0.177 ± 0.005[ijk]
	75	Green	0.199 ± 0.006[hi]
		Purple	0.183 ± 0.005[hijk]
	90	Green	0.205 ± 0.006[gh]
		Purple	0.190 ± 0.005[hij]

*Least square mean values ± standard error (n = 12 for Purple cultivar, n = 9 for Green cultivar) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table C.10. Least square means of percentage of specific methane yield of selected samples of plant parts of the Energycane at different incubation time

Plant parts	Incubation time (days)	Percentage of specific methane yield (90 day's yield as final yield)
Leaf, Bottom	15	59.77 ± 0.58^{o}
	30	82.23 ± 0.58^{l}
	35	85.76 ± 0.58^{j}
	45	85.76 ± 0.58^{j}
	60	90.49 ± 0.58h^{i}
	75	94.08 ± 0.58^{defg}
Leaf, Top	15	62.58 ± 0.58^{n}
	30	85.38 ± 0.58^{jk}
	35	87.68 ± 0.58^{ij}
	45	87.68 ± 0.58^{ij}
	60	92.20 ± 0.58^{fgh}
	75	95.00 ± 0.58^{cdef}
Stem, Bottom	15	82.42 ± 0.58^{kl}
	30	92.97 ± 0.58^{efgh}
	35	94.26 ± 0.58^{cdefg}
	45	94.26 ± 0.58^{cdefg}
	60	96.38 ± 0.58^{bcd}
	75	97.77 ± 0.58^{ab}
Stem, Top	15	78.61 ± 0.58^{m}
	30	91.57 ± 0.58^{gh}
	35	92.72 ± 0.58^{efgh}
	45	92.72 ± 0.58^{efgh}
	60	95.37 ± 0.58^{bcde}
	75	96.94 ± 0.58^{abc}

*Least square mean values ± standard error (n = 6) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table C.11. Least square means of percentage of specific methane yield of selected samples of plant parts of the Energycane cultivars at different incubation time

Plant part	Incubation time (days)	Cultivar	Percentage of specific methane yield (90 day's yield as final yield)
Leaf, Bottom	15	77-9271	55.98 ± 0.78^{t}
		MOL 6136	63.55 ± 0.78^{rs}
	30	77-9271	80.93 ± 0.78^{opq}
		MOL 6136	83.53 ± 0.78^{lmnop}
	35	77-9271	85.68 ± 0.78^{lmn}
		MOL 6136	85.83 ± 0.78^{klmn}
	45	77-9271	85.68 ± 0.78^{lmn}
		MOL 6136	85.83 ± 0.78^{klmn}
	60	77-9271	90.37 ± 0.78^{hijk}
		MOL 6136	90.61 ± 0.78^{ghij}
	75	77-9271	94.09 ± 0.78^{bcdefgh}
		MOL 6136	94.06 ± 0.78^{bcdefgh}
Leaf, Top	15	77-9271	59.58 ± 0.78^{st}
		MOL 6136	65.58 ± 0.78^{r}
	30	77-9271	84.78 ± 0.78^{lmno}
		MOL 6136	85.97 ± 0.78^{klmn}
	35	77-9271	87.26 ± 0.78^{jklm}
		MOL 6136	88.10 ± 0.78^{ijkl}
	45	77-9271	87.26 ± 0.78^{jklm}
		MOL 6136	88.10 ± 0.78^{ijkl}
	60	77-9271	92.26 ± 0.78^{dfghi}
		MOL 6136	92.14 ± 0.78^{dfghi}
	75	77-9271	95.12 ± 0.78^{bcdefg}
		MOL 6136	94.88 ± 0.78^{bcdefgh}

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table C.11. (Continued) Least square means of percentage of specific methane yield of selected samples of plant parts of the Energycane cultivars at different incubation time

Plant part	Incubation time (days)	Cultivar	Percentage of specific methane yield (90 day's yield as final yield)
Stem, Bottom	15	77-9271	81.81 ± 0.78^{nopq}
		MOL 6136	83.03 ± 0.78^{mnop}
	30	77-9271	92.91 ± 0.78^{cdefgh}
		MOL 6136	93.02 ± 0.78^{cdefgh}
	35	77-9271	94.10 ± 0.78^{bcdefgh}
		MOL 6136	94.43 ± 0.78^{bcdefgh}
	45	77-9271	94.10 ± 0.78^{bcdefgh}
		MOL 6136	94.43 ± 0.78^{bcdefgh}
	60	77-9271	96.52 ± 0.78^{abce}
		MOL 6136	96.25 ± 0.78^{abcde}
	75	77-9271	97.87 ± 0.78^{ab}
		MOL 6136	97.67 ± 0.78^{ab}
Stem, Top	15	77-9271	79.04 ± 0.78^{pq}
		MOL 6136	78.19 ± 0.78^{q}
	30	77-9271	92.19 ± 0.78^{defghi}
		MOL 6136	90.95 ± 0.78^{fghij}
	35	77-9271	93.36 ± 0.78^{bcdefgh}
		MOL 6136	92.09 ± 0.78^{defghi}
	45	77-9271	93.36 ± 0.78^{bcdefgh}
		MOL 6136	92.09 ± 0.78^{defghi}
	60	77-9271	95.86 ± 0.78^{abcde}
		MOL 6136	94.88 ± 0.78^{bcdef}
	75	77-9271	97.18 ± 0.78^{abc}
		MOL 6136	96.69 ± 0.78^{abcde}

*Least square mean values ± standard error (n = 3) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table C.12. Least square means of percentage of specific methane yield of selected samples of plant parts of the Napier grass at different incubation time

Plant part	Incubation time (days)	Percentage of specific methane yield (90 day's yield as final yield)
Leaf	15	62.86 ± 0.50^{g}
	30	82.59 ± 0.50^{f}
	35	85.37 ± 0.50^{e}
	45	89.17 ± 0.50^{d}
	60	92.88 ± 0.50^{c}
	75	96.26 ± 0.50^{b}
Stem	15	65.22 ± 0.50^{g}
	30	83.84 ± 0.50^{ef}
	35	86.20 ± 0.50^{e}
	45	90.15 ± 0.50^{d}
	60	93.54 ± 0.50^{c}
	75	96.63 ± 0.50^{b}

*Least square mean values ± standard error (n = 21) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

Table C.13. Least square means of percentage of specific methane yield of selected samples of plant parts of the Napier grass cultivars at different incubation time

Plant part	Incubation time (days)	Cultivar	Percentage of specific methane yield (90 day's yield as final yield)
Leaf	15	Green	62.31 ± 0.77^{l}
		Purple	63.40 ± 0.63^{l}
	30	Green	82.60 ± 0.77^{j}
		Purple	82.59 ± 0.63^{j}
	35	Green	85.45 ± 0.77^{hij}
		Purple	85.29 ± 0.63^{hij}
	45	Green	89.75 ± 0.77^{efg}
		Purple	88.58 ± 0.63^{gh}
	60	Green	93.21 ± 0.77^{cdef}
		Purple	92.54 ± 0.63^{def}
	75	Green	96.65 ± 0.77^{abc}
		Purple	95.87 ± 0.63^{cd}
Stem	15	Green	61.31 ± 0.77^{l}
		Purple	69.14 ± 0.63^{k}
	30	Green	82.85 ± 0.77^{j}
		Purple	84.82 ± 0.63^{ij}
	35	Green	85.34 ± 0.77^{hij}
		Purple	87.06 ± 0.63^{ghi}
	45	Green	90.26 ± 0.77^{efg}
		Purple	90.03 ± 0.63^{fg}
	60	Green	93.63 ± 0.77^{cdef}
		Purple	93.45 ± 0.63^{cde}
	75	Green	96.89 ± 0.77^{abc}
		Purple	96.37 ± 0.63^{bc}

*Least square mean values ± standard error (n = 12 for Purple cultivar, n = 9 for Green cultivar) followed by a different letter within a column are significantly different at the $p \leq 0.05$ level

APPENDIX D: LIST OF PUBLICATIONS

Under Preparation

Surendra, K.C., Ogoshi, R., Zaleski, H., Hashimoto, A., Khanal, K. High yielding tropical energy crops for bioenergy production: Effects of plant components, harvest years, and locations on biomass composition.

Surendra, K.C., Ogoshi, R., Reinhardt-Hanisch, A., Oechsner, H., Zaleski, H., Hashimoto, A., Khanal, K. Anaerobic digestion of high yielding tropical energy crops for biogas production: Effects of crop types, locations and plant parts.

Published

Surendra, K.C., Olivier, R., Tomberlin, J.K., Jha, R., Khanal, S.K., 2016. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. *Renewable Energy* 98, 197-202.

Sawatdeenarunat, C., Nguyen, D., **Surendra, K.C.,** Shrestha, S., Rajendran, K., Oechsner, H., Xie, L., Khanal, S.K., 2016. Anaerobic biorefinery: Current status, challenges, and opportunities. *Bioresource Technology* 215, 304-313.

Surendra, K.C., Sawatdeenarunat, C., Shrestha, S., Sung, S., Khanal, S.K., 2015. Anaerobic digestion-based biorefinery for bioenergy and bio-based products. *Industrial Biotechnology* 11, 103-112.

Sawatdeenarunat, C., **Surendra, K.C.,** Takara, D., Oechsner, H., Khanal, S.K., 2015. Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. *Bioresource Technology* 178, 178-86.

Surendra, K.C., Khanal, S.K., 2015. Effects of crop maturity and size reduction on digestibility and methane yield of dedicated energy crop. *Bioresource Technology* 178, 187-93.

Surendra, K.C., Takara, D., Hashimoto, A.G., Khanal, S.K., 2014. Biogas as a sustainable energy source for the developing countries: Opportunities and challenges. *Renewable and Sustainable Energy Reviews* 31, 846-59.

Takara, D., Nitayavardhana, S., Munasinghe, P., **Surendra, K.C.,** Khanal, S.K., 2012. Sustainable bioenergy from biofuel-derived residues. *Water Environment Research* 84, 1568-1585.

Surendra, K.C., Khanal, S.K., Shrestha, P., Lamsal, B., 2011. Current status of renewable energy in Nepal: Opportunities and challenges. *Renewable and Sustainable Energy Reviews* 15, 4107-4117

PRESENTATION IN CONFERENCES

International

Surendra, K.C., Ogoshi, R., Reinhardt-Hanisch, A., Oechsner, H., Hashimoto, A., Khanal, S.K.* High yielding tropical energy crops for bioenergy production: Effects of plant components, harvest years, and locations on biomass composition and subsequent biogas production. International Conference Progress in Biogas IV (Mar 8-11, 2017), Stuttgart, Germany (Podium presentation)

Surendra, K.C., Olivier, R., Tomberlin, J.K., and Khanal, S.K. Insect farming on organic wastes: A novel strategy to produce biodiesel and animal feed with concurrent waste remediation. International Conference on Challenges in Environmental Science and Engineering (Sep 28-Oct 2, 2015), Sydney, Australia (Podium presentation)

Surendra, K.C., and Khanal, S.K. Effect of crop maturity stage and size reduction on digestibility of energy crop for biomethane production by anaerobic digestion. American Society of Agricultural and Biological Engineers (ASABE) 2014 Annual International Meeting (July 13-16, 2014), Montreal, QC, Canada (Podium presentation)

National

Surendra, K.C., Olivier, R., Tomberlin, J.K., and Khanal, S.K. Bioconversion of food wastes to biodiesel and animal feed through insect farming. 27th Annual CTAHR and Student Research Symposium (Apr 10-11, 2015), University of Hawai'i at Mānoa, Honolulu, HI, USA (Poster presentation)

Surendra, K.C., and Khanal, S.K. Effect of crop maturity and size reduction on digestibility of energy crop for biomethane production. 26th Annual CTAHR and Student Research Symposium (Apr 11-12, 2014), University of Hawai'i at Mānoa, Honolulu, HI, USA (Podium presentation)

Surendra, K.C., and Khanal, S.K.* Ensilage strategy to pretreat green grass for enhanced biomethane production. 27th annual Biocycle West Coast Conference 2013 (Apr 9-11, 2013), San Diego, California, USA (Podium presentation)

Surendra, K.C., Hashimoto, A.G., and Khanal, S.K. Biological ensilage additives as pretreatment for anaerobic digestion of green grass for enhanced biomethane production. 24th Annual CTAHR Student Research Symposium (Apr 13-14, 2012), University of Hawai'i at Mānoa, Honolulu, HI, USA (Poster presentation)

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